The Journal of
CLINICAL ENDOCRINOLOGY
AND METABOLISM

VOLUME 19 JULY, 1959 NUMBER 7
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SULFATION FACTOR ACTIVITY OF SERA FROM PATIENTS WITH PITUITARY DISORDERS*

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ABSTRACT

Previous work from this laboratory established the fact that normal human and rat serum contains a factor which stimulates the *in vitro* incorporation of sulfate-S^{35} into cartilage from hypophysectomized rats. This activity of serum which appears to be due to a protein or protein-bound component is not attributable to the presence of unaltered growth hormone. An assay for sulfation factor in human serum is described in this paper. The serum from normal subjects was found to contain from 0.5 to 1.5 times the activity of a standard reference serum in nearly all cases. No significant change in sulfation factor activity with age could be demonstrated. Decreased sulfation factor activity was present in the sera of patients with spontaneous hypopituitarism or panhypopituitarism following hypophysectomy. The sulfation factor activity was increased in the serum of 5 patients with acromegaly early in the course of the disease but later, when the disease was inactive, the concentration of sulfation factor was generally normal. It is concluded that the measurement of serum sulfation factor

Received January 21, 1959.

* Presented at the Fortieth Meeting of The Endocrine Society, San Francisco, California, June 19–21, 1958.

This investigation was supported by research grant A-1526, from the National Institute of Arthritis and Metabolic Diseases, Bethesda, Maryland; and a grant from The Arthritis and Rheumatism Foundation, St. Louis, Missouri.

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activity provides an index of pituitary activity in respect to growth, permitting a more accurate evaluation of endocrine-induced disturbances of growth.

INTRODUCTION

ANALYSIS of the humoral regulation of growth has been hampered by the absence of reliable means of quantitating the amount of the circulating growth-promoting substances in the blood. Attempts to adapt the tibial-line assay for growth hormone to the measurement of this hormone in human serum have yielded varying results. Kinsell and associates (1) obtained evidence of tibial-line stimulation by lyophilized plasma from a patient with acromegaly. Gemzell and co-workers (2) also noted tibial-line stimulating activity in acromegalic serum, but could not detect this activity in normal human plasma or plasma fractions. Using a similar method, Segaloff *et al.* (3) obtained evidence of a surprising concentration of a growth hormone-like material in normal human plasma—the equivalent of 0 to 40 μg. per ml. Such activity was not present in plasma from patients with panhypopituitarism, and increased concentrations (41, 61 and 55 μg. per ml.) were found in the plasma of 3 patients with active acromegaly. Despite the fact that the reported results are consistent with the clinical situations studied, there is reason to doubt the accuracy and specificity of the test systems. Moreover, the large number of animals required for even limited accuracy precludes the use of this method for large-scale clinical investigative use.

A most promising development in the search for a method of measuring growth hormone in plasma has been the development of immunologic methods using anti-human-growth-hormone serum. It would appear from the initial results with this approach that the concentration of immunologically detectable growth hormone in human serum is from 0.24 to 0.64 μg. per ml. (4), which is much less than that reported by Segaloff and co-workers.

In this laboratory, interest has been centered on the hormonal regulation of cartilage metabolism. The incorporation of sulfate-S^{35} into the rat's tibial epiphysis *in vivo* is markedly impaired by hypophysectomy of the young animal, and this defect can be promptly corrected by treatment with small amounts of pituitary growth hormone (5). Although this index of growth hormone action proved to be a sensitive assay for purified hormone preparations, the results with human serum proved unreliable in our laboratory as well as in others (6). Attention was therefore directed to the development of an *in vitro* system in which the humoral activation of chondroitin sulfate synthesis in cartilage from hypophysectomized rats could be examined. In a suitable incubation system, normal rat serum was capable of activating sulfation by cartilage from hypophysectomized rats

(7). This property was consistently absent from the serum of hypophysectomized rats, but returned in the serum of such animals after administration of growth hormone. Evidence has been presented that the factor in normal serum which has been called "sulfation factor" is not related to glutamine or other dialyzable components of the serum. When bovine (7) growth hormone is added *in vitro* it does not elicit a stimulation of sulfation comparable to that elicited by human growth hormone. Some stimulation of sulfation is evident when large concentrations of insulin are tested by the *in vitro* system, but physiologic concentrations of insulin do not affect cartilage sulfation. The exact relationship between growth hormone and sulfation factor remains undefined.

Preliminary experiments demonstrated the presence of sulfation factor activity in human serum (9). In this paper an assay for human sulfation factor is described, and results obtained with this assay using serum from normal subjects and patients with pituitary disease are presented.

METHODS

Material. Blood was collected without addition of anticoagulants. The serum was separated from the clot, divided into convenient portions, and frozen in a deep-freeze at −14° C. No change in sulfation factor activity was detected after many months of storage in the frozen state. Serum samples collected in other centers were packed in dry ice and shipped by air.

The human growth hormone used in certain experiments was made from a pool of human pituitaries which had been collected in acetone and processed by Dr. Maurice Raben of Boston. The growth hormone powder was dissolved in 0.01 *N* hydrochloric acid, to which penicillin was added. No adverse reactions were noted to subcutaneous injection of growth hormone, other than slight nausea in 2 patients and trivial local reactions.

Conditions of assay. Details of the incubation technique have been described (8, 10). Costal cartilages were obtained from rats hypophysectomized ten to twenty days previously at an age of about 25 days. Ten costal cartilage segments were taken from each of 8 rats, carefully trimmed free of soft tissue and bone, and distributed at random among 10 treatment groups.

The incubations were conducted in individual glass vials containing 0.5 ml. of a medium that contained the test serum, amino acids and buffer salts. When all dissections were completed, 0.2 ml. of a solution containing sulfate-S^{35}, glucose, antibiotics and buffer was added to each vial.

A pool of serum obtained from a normal young man, 24 years of age, was established as a reference standard for comparison. The promotion of sulfation by the reference serum was measured with each assay to be reported, because there was considerable variation in the degree of stimulation produced by the normal serum from experiment to experiment. At first the reference serum was tested at only a single concentration (0.1 ml. per vial) but, in order to improve accuracy, reference serum was later tested at three dilutions (0.1, 0.05 and 0.017 ml. per vial). Unknown sera were examined at only one level (0.1 ml. per vial) in most of the assays, but in the studies with human growth hormone conducted on Subjects J.R., D.M. and N.H., and in certain other critically important assays, two or more levels of serum were tested.

The cartilage segments were incubated for twenty-four hours at 37° C. in an atmosphere of air. At the end of this period each cartilage was placed in an individual chamber of a specially designed washing and drying rack. The rack was made from an aluminum plate, $\frac{3}{8}$ inch in thickness, in which 80 holes, each 1.1 cm. in diameter, had been drilled. A fine stainless-steel screen was clamped to the bottom of the plate to provide the floor of the chambers. A second reinforced stainless-steel screen was bolted to the top of the plate after the cartilages had been placed in their proper chambers. The support for the upper screen had sides which rose 2.2 cm. above the screen to form a trough which aided in washing the cartilage segments.

Washing of the cartilage segments was started with boiling water and continued with cold water for three hours in order to remove unesterified sulfate. The cartilage segments, still in the same rack, were dried under an infrared lamp and weighed on a Cahn electrobalance to the nearest 0.01 mg. The dry weight of each cartilage varied from about 1 mg. to 3 mg. The cartilages were then placed in 12-ml. conical centrifuge tubes containing 0.4 ml. of 6 *N* hydrochloric acid. The tubes, stoppered with a single-holed rubber stopper, were heated in a steam bath for three hours. The cartilage hydrolysates were transferred directly to polyethylene cups (2.6 cm. in diameter) with a capillary pipette, using two 0.2-ml. water washes. The water and acid were evaporated under an infrared lamp and the cups were counted for radioactivity in the Geiger range by a gas flow detector with a Micromil window. At least 2000 counts per cup were made. The self absorption amounted to only about 7 per cent in these experiments, and this value was used to correct all the results.

In addition to knowing the amount of sulfate-S^{35} taken up in these experiments it was necessary to know the specific activity of the incubation medium. The sulfate pool in the incubation medium was calculated from the concentration of sulfate in the added phosphosaline buffer and the concentration of sulfate was determined in the serum (10). On the basis of specific activity of the sulfate in the medium, the total sulfate uptake in micrograms (μg.) per 100 mg. of cartilage was calculated. The method of least squares was used to calculate the regression of sulfate uptake on the logarithm of the amount of reference serum added (Fig. 1). The potency of the reference serum was arbitrarily established as 1.0, and the potency of unknown serum was calculated using standard methods. Because the lowest points on the standard dose-response curve were near the threshold of sensitivity of the cartilage, the slopes observed with sera from patients with hypopituitarism were much flatter than those with the reference serum. In this situation, only the values which fell within the lower effective range of the assay were used to calculate potency. At the other extreme of activity, single-point assays on patients with acromegaly often yielded sulfate uptakes considerably beyond the range of the dose-response curve with reference serum. The potency values obtained by extrapolation were therefore subject to enhanced error.

In the course of these measurements it became necessary to establish a second pool of reference serum. Two assays were conducted with 3 concentrations of each serum so that the activity of the second serum could be expressed in terms of the first. These experiments also provided an opportunity to analyze the statistical validity of the assay using the methods of Bliss and Calhoun (11). The logarithm of the potency and the standard error of the logarithm of potency ($M \pm S_M$) were found to be 0.1034 ± 0.08 and 0.0297 ± 0.07, which corresponds to an actual potency of 1.27 and 1.11. The index of precision, λ, was found to be 0.25 and 0.28 in the 2 experiments.

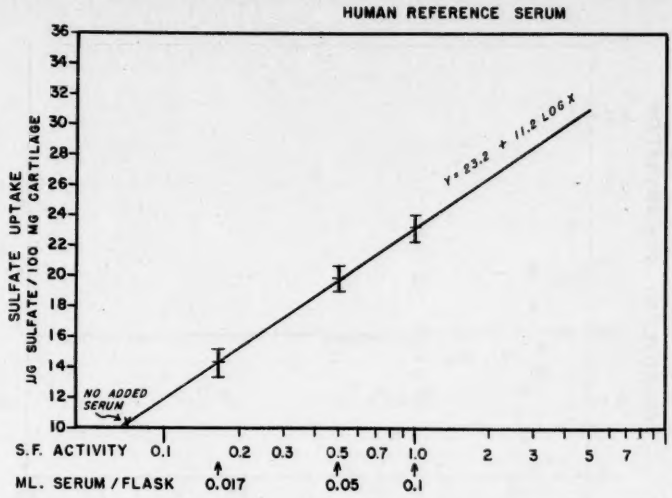

FIG. 1. Effect of 3 *in vitro* additions of normal human serum on the sulfate uptake by cartilage from hypophysectomized rats. The gradients represent ± the standard error of the mean.

RESULTS

Normal subjects

Sulfation factor activity was determined in sera from 29 persons without pituitary disease, between the ages of 7 years and 73 years. Sulfation factor was found in the sera at all ages (Fig. 2). Moreover, there was only an insignificant apparent decline in the mean value with age. The mean potency and standard error for 11 sera from children between the ages of 7 and 12 years as compared to the reference serum was 0.84 ± 0.12; for 14 sera from adults 20 to 40 years old, 0.78 ± 0.13; and for 5 sera from adults more than 50 years old, 0.75 ± 0.12. Most of the results were in the range of 0.5 to 1.5. Included in Figure 2 are the results of sulfation factor measurements on the sera from 5 pregnant women; the mean potency was 0.84, which is not significantly different from that for either men or women of the same age.

Sulfation factor activity was determined five times during the day in 3 young men (Fig. 3). Meals were eaten at the customary hours, and the men continued their normal activities as students. The results for any one subject were quite stable throughout the day, and no consistent diurnal pattern could be detected. From these observations it appears that the practice of obtaining blood in the midmorning or midafternoon introduces no particular bias.

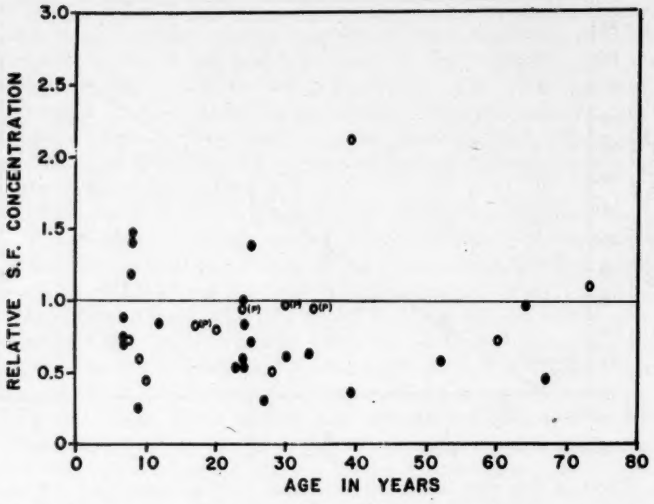

FIG. 2. Sulfation factor activity in sera from 34 persons without recognizable pituitary disease expressed as the potency relative to the normal reference serum. Assays on sera from men and boys are plotted as (•), on sera from women and girls as (o); and on serum from a pregnant woman as (o(P)).

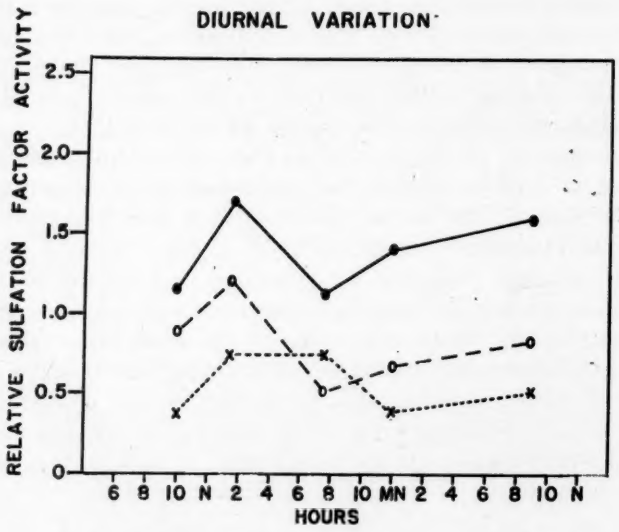

FIG. 3. Sulfation factor activity at different times of the day. in the serum of 3 normal men.

TABLE 1. SULFATION FACTOR ACTIVITY OF SERUM IN HYPOPITUITARISM (EXCLUDING CASES OF HYPOPITUITARISM DUE TO PITUITARY TUMOR)

I. *Pituitary dwarfism*

Case No.	Patient	Age (yrs.)	Sex	Height (cm.)	S. F. activity*
1	N.H.	7½	F	79	0.16
2	L.H.	15	F	133	0.24
3	W.M.**		M		0.08
4	J.R.	26	M	135	0.09

II. *Sheehan's syndrome* (postpartum necrosis of the pituitary)

Case No.	Patient	Age (yrs.)	Time since last pregnancy	S. F. activity
1	M.R.	19	10 mos.	0.24
2	R.L.	19	3 yrs.	0.12
3	M.†	56	15 yrs.	0.19
4	I.S.	40	23 yrs.	0.12
5	L.C.	45	25 yrs.	0.27

III. *Post-hypophysectomy*

Case No.	Patient	Sex	Indication for surgery	S. F. activity
1	H.	F	Cancer of breast	0.17
2	M.T.	F	Cancer of breast	0.06
3	A.D.*	F	Cancer of breast	0.10
4	J.H.*	F	Cancer of breast	0.26
5	A.C.*	F	Cancer of breast	0.10
6	R.W.*	F	Cancer of breast	0.09
7	A.F.*	M	Cancer of prostate	0.08
8	L.B.*	M	Cancer of prostate	0.08
9	R.T.*	M	Diabetic retinopathy	0.08

* Potency as compared to reference serum.
** Serum provided by Dr. Olaf Pearson.
† Serum provided by Dr. Maurice Raben.

Hypopituitarism

The consistent absence of sulfation factor activity in the serum of hypophysectomized rats was the key observation of our previous work (8). It was of the utmost importance to examine the serum of patients with hypopituitarism in order to demonstrate that the material which we were finding in human serum was comparable to that in the rat. Sulfation factor assays on 18 patients with 3 types of hypopituitarism are presented in Table 1. The diagnosis in the 4 cases of pituitary dwarfism was based on

the combination of marked retardation of growth with evidence of impaired secretion of other pituitary hormones. Some of the patients were receiving desiccated thyroid and gonadal and adrenal steroids, separately or in combinations, at the time of study. The 5 patients with Sheehan's syndrome all gave a history of an obstetrical catastrophe followed by evidence of gonadal, thyroid, and, in some cases, adrenal hypofunction. The sulfation factor activity was found to be significantly low in this group of patients.

To answer the question, "What is the sulfation factor activity in the absence of pituitary function?," we examined the sera from 9 patients who had been hypophysectomized—8 because of metastatic carcinoma, and 1 because of diabetic complications. Two of the patients had operations at Barnes Hospital and the remainder at the Memorial Center in New York, where there has been extensive experience with this operation. With 1 possible exception, the level of sulfation factor in this group of patients was extremely low; moreover, there was little assurance that this activity was due to the same material that is present in serum from normal persons.

Pituitary neoplasms

The results of sulfation factor measurements in patients with pituitary tumors indicate a wide range of values (Table 2A). Sulfation factor activity was low in the 2 patients with craniopharyngioma, and postopera-

TABLE 2A. SULFATION FACTOR ACTIVITY OF SERUM IN PITUITARY NEOPLASIA

Case No.	Patient	Age & Sex	Clinical & laboratory evidence of dysfunction*				Status of treatment	S.F. activity of serum†
			TSH	ACTH	Gonadotropins	Diabetes insipidus		
			Craniopharyngioma					
1	D.M.	8 F	↓ (Rx)	↓ (Rx)	↔Pregnant	++(Rx)	Postop.	0.32
2	M.R.	20 F	↔	↔		0	None	0.81
			Chromophobe adenoma					
1	A.D.	22 M	↓ (Rx)		↓ (Rx)	0	Postop.	0.08
2	B.W.	36 F	↓ (Rx)	↔	↓	+(transient)	Postop.	0.21
3	J.W.	51 M	↓	↓	↓	+(transient)	Postop.	1.03
4	B.G.	38 F	↓	↔	↓	0	Preop.	0.80
							Postop.	0.15
5	G.Z.	54 M	↓ (Rx)	↓ (Rx)	(Rx)	+	Postop.	0.72
6	R.McC.	39 F	↔	↔	↓ (lactation)	0	None	0.94
7	E.O.	43 M	↓ (Rx)	↓	↓ (Rx)	0	X-ray	0.26
8	A.	70 M	↓ (Rx)	↓ (Rx)	↓ (Rx)	0	Postop.	0.27

* (Rx) indicates that the patient was receiving replacement treatment at the time of study.
→ indicates evidence of decreased end-organ function.
↔ indicates no evidence of altered end-organ function.
† Potency as compared to reference serum.

tively in 2 of the 8 patients with chromophobe adenoma. It was doubtful that all neoplastic tissue had been removed in most cases. The table also presents some information about other aspects of pituitary function. Two of the patients with chromophobe adenoma (G.Z. and J.W.) had unequivocal evidence of decreased thyroid and gonadal function, yet had normal levels of sulfation factor as measured by this method. The implications of this observation are discussed in a later section.

The sulfation factor activity of serum from 16 patients with acromegaly has been recorded in Table 2B. The variable clinical activity of the disease makes it important to consider certain clinical parameters in relation to the level of sulfation factor in acromegalic patients. The duration of acromegaly is difficult to establish because of the insidious onset. A short duration of symptoms is presumptive evidence of active disease, a phase probably decreased by x-ray or surgical treatment. Our assessment of clinical

TABLE 2B. SULFATION FACTOR ACTIVITY OF SERUM IN EOSINOPHIL ADENOMA (ACROMEGALY)

Case No.	Patient	Age & Sex	Acromegaly			Serum	
			Duration (yrs.)	Treatment	Activity	Phos. (mg./100 ml.)	S.F. activity*
1	M.B.	18 F	1	None	+	5.6	1.6
2	R.	37 F	3	None	++	—	3.7
3	G.B.	33 F	3½	None	++	4.6	6.0
4	E.S.	36 F	4	None	+++	6.1	8.2
5	H.Z.	58 F	5	None	+++	—	6.4
6	M.McC.	54 F	15	None	++	5.0	4.4
7	D.B.**	62 F	20	None	0	4.3	0.7
8	W.P.	46 M	20	None	++	4.7	1.3
9	W.L.†	62 M	20	None	0	3.9	0.9
10	L.B.	53 F	20	X-ray (2 courses)	0	3.8	7.8 0.5
11	M.S.	38 F	6	X-ray	§	—	0.9
12	R.G.	35 M	6	X-ray (2 courses)	+	4.2	1.0
13	E.A.	37 F	8	X-ray	0	4.0	7.8
14	R.F.	63 M	10	X-ray	0	3.2	1.3
15	C.P.	47 M	12	X-ray	0	—	1.2
16	L.B.	41 F	17	X-ray	0	3.8	1.6
17	E.R.	53 F	26	X-ray	+	4.7	1.9

* Potency as compared to reference serum.

** Serum provided by Dr. Olaf Pearson.

† Serum provided by Dr. Maurice Raben.

§ Too soon after x-ray treatment for clinical evaluation.

activity (Table 2) was an impression based on the pool of historical, laboratory and x-ray evidence which indicated continuous overproduction of growth hormone. Because serum for sulfation factor measurements was not obtained during fasting, inorganic phosphate determinations were made at a different time; these are included for 13 of the 17 cases listed in Table 2B.

In 4 acromegalic patients who were untreated and examined early in the course of the illness, the levels of serum sulfation factor were increased; this occurred also in 1 other untreated acromegalic whose disease had been present for a longer period. In Patient W.P. the disease was considered to be active on clinical grounds, yet sulfation factor activity was normal in 2 serum specimens. Sulfation factor levels became elevated early in the course of x-ray treatment, suggesting increased release of growth hormone.

In most of the patients with long-standing, inactive disease treated by one or more courses of x-ray therapy, sulfation factor activity was normal. The high levels found in 1 patient could not be confirmed on subsequent analysis. Definite elevation was observed in 1 patient, and doubtful elevation in 2 others.

The course of therapy was followed with repeated assays in 2 patients with acromegaly. Patient G.B. was 35 years old at the time the first sulfation factor activity measurement was made. She had had evidence of the disease for only three years. Intensive treatment with the betatron was applied to the pituitary over the period of a month. Serum sulfation factor activity was high before treatment and subsided slowly after treatment, but had not reached normal levels within five months (Fig. 4).

Patient E.S. was 36 years old when she was seen at the Washington University Clinics because of amenorrhea and persistent lactation for four years. Enlargement of the hands and feet had been recognized for only two years. Some clinical improvement followed x-ray treatment to the pituitary, but progressive encroachment on the visual fields prompted surgical attack on the tumor. As shown in Figure 5, there was a high initial level of serum sulfation factor activity and suggestive evidence of subsidence after x-ray therapy; the erratic changes following operation may or may not represent a real phenomenon. Subsequently there has been a return to normal values for sulfation factor.

Effects of primate growth hormone on serum sulfation factor activity

Sera before and after the administration of human growth hormone to a patient hypophysectomized because of metastatic carcinoma were obtained from Dr. Olaf Pearson, and serum from 2 patients with hypopituitarism who had been given monkey growth hormone were provided by Dr. Maurice Raben. The results of sulfation factor assays are presented in Fig-

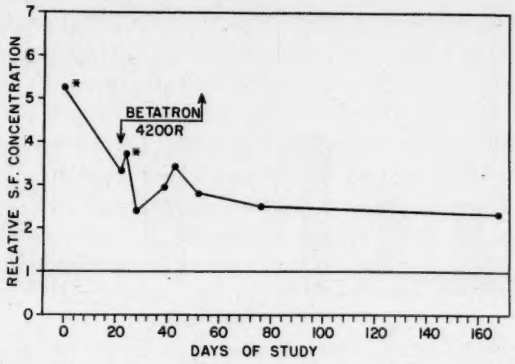

FIG. 4. Sulfation factor activity of serum in a patient with acromegaly (G.B.) before and after treatment of the pituitary by the betatron. Results marked with an asterisk represent the mean of 2 assays.

ure 6. Suggestive rises in sulfation factor activity were observed in all subjects and prompted a more thorough study.

The effect of growth hormone on sulfation factor activity was therefore studied in 3 patients with pituitary dwarfism. One had a craniopharyngioma; in the other 2, the etiology of the hypopituitarism was presumably non-neoplastic. The first patient, J.R., was 26 years old at the time of study. Although his growth as an infant was said to have been normal, it became obviously retarded after the age of 4 years. He remained sexually infantile. Signs of hypothyroidism were not clearly evident despite the fact that the plasma protein-bound iodine level was 1.8 μg. per 100 ml. No impairment of general health and vigor had been noted, yet 17-hydroxycorticosteroid ex-

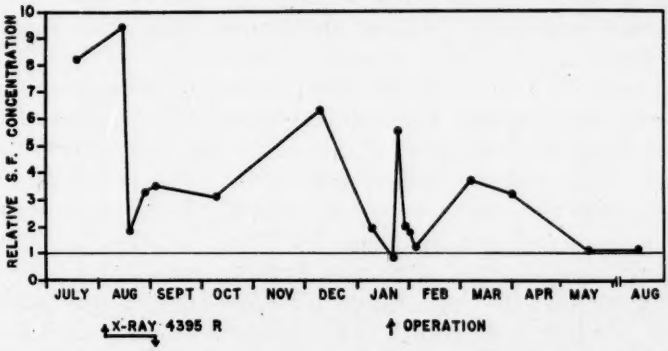

FIG. 5. Sulfation factor activity of serum in a patient with acromegaly (E.S.) before and after treatment of the pituitary by the betatron and after surgical removal of an eosinophilic adenoma.

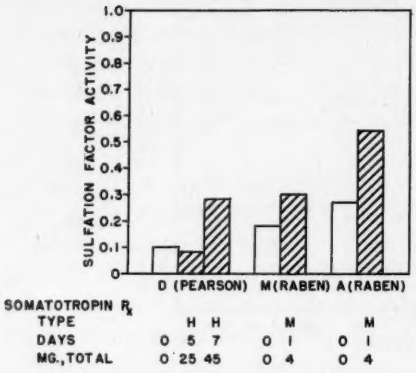

FIG. 6. Sulfation factor activity of serum in 3 patients with hypopituitarism before and after treatment with either human (somatotropin type H) or monkey (somatotropin type M) growth hormone. (Sera provided by Dr. M. Raben and Dr. O. Pearson.)

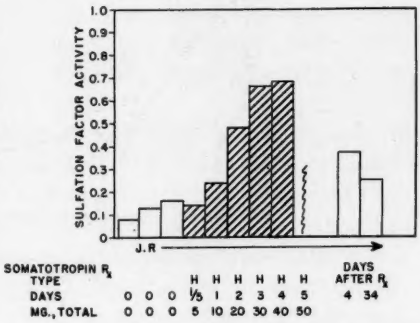

FIG. 7. Sulfation factor activity in the serum of Patient J.R. (pituitary dwarfism) before and after the administration of human growth hormone.

cretion in the urine was only 0.8 mg. per day. No urinary gonadotropin was found in a 24-hour specimen. Assays of serum sulfation factor (Fig. 7) demonstrated a progressive rise during the four days of treatment with growth hormone. It would appear that the return to this patient's characteristic low levels would require more than thirty days.

The second patient, D.M., was 9 years of age at the time of study. She was born after a difficult delivery and was slow to begin spontaneous breathing. Later it was noted that her rate of growth was slow. Because of vomiting and progressive physical and mental retardation she was referred to the St. Louis Children's Hospital. Enlargement of the sella turcica was demonstrated. Two operations were performed to remove what proved to be a craniopharyngioma. Postoperatively permanent diabetes insipidus developed. Medication consisted of thyroid (60 mg. daily), cortisone (12.5 mg. twice a day), and insufflations of posterior pituitary powder. The response to growth hormone is charted in Figure 8. There was an unequivocal increase in serum sulfation factor activity. The initial baseline values were higher than those observed after total hypophysectomy for cancer. It is also of interest that the dose of growth hormone, which was higher than needed to stimulate good growth in hypophyseal dwarfs, raised the sulfation factor activity of one specimen of serum to more than twice the mean normal level.

The third patient, N.H., was $7\frac{1}{2}$ years of age at the time of study. Al-

though she weighed 2600 Gm. at birth, she was noted to be short. Her subsequent rate of growth was decidedly slow. Her weight at the time of study was 21 pounds 13 ounces, height 33¼ inches, and bone age only 3 years. Urinary 17-hydroxycorticosteroid excretion was 1.6 mg. per day. The changes in serum sulfation factor activity following the administration of human growth hormone are presented in Figure 9. After two days of treatment, a barely significant elevation was demonstrable, but at the completion of five days of treatment the sulfation factor activity had risen from 0.16 to 0.78 as compared to the reference serum.

DISCUSSION

In considering the effects of serum on cartilage, it is helpful to make a distinction between the components of serum which provide nutrients for the cartilage cells and the hormonal materials which regulate, rather than take part in the synthetic processes. In our studies, glucose and amino acids were added to the incubation medium so that the small amounts of these materials contributed by the serum would not significantly influence sulfate uptake. From previous experiments (10) it was known that biotin, folic acid, nicotinamide, pantothenate, pyridoxal, riboflavine, thiamin, inositol, choline, glutamine and glucosamine do not influence the degree of sulfate uptake by cartilage under the conditions of these incubations; so these materials were not added. There is evidence that in serum there are as yet uncharacterized dialyzable components which are not represented in the basal medium but which influence the assay. The slight stim-

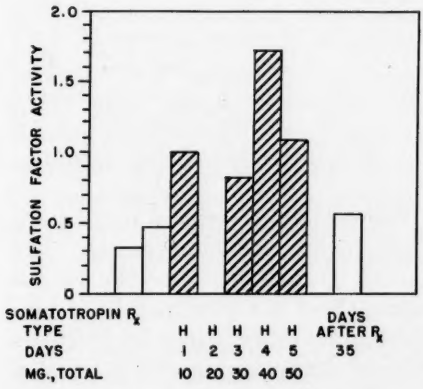

FIG. 8. Sulfation factor activity in serum of Patient D.M. (dwarfism due to a craniopharyngioma) before and after the administration of human growth hormone.

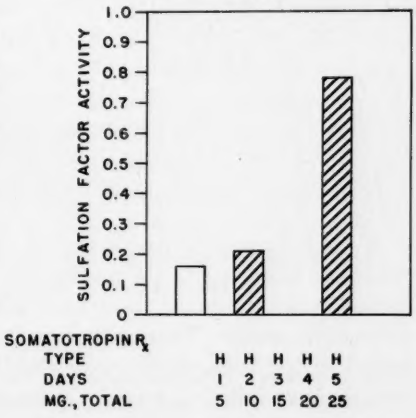

FIG. 9. Sulfation factor activity in serum of Patient N.H. (pituitary dwarf) before and after the administration of human growth hormone.

ulation of sulfation consistently found in the serum of hypophysectomized patients may be due to such factors.

The major stimulatory effect of normal serum appears to be of a hormone-dependent nature. The dependence of sulfation factor on pituitary function and more specifically on the growth hormone has been established in man by these observations and corresponds in all essential features to the condition existing in the rat. The distinction between sulfation factor and growth hormone merits restatement. It is based on two types of evidence: 1) the inactivity of growth hormone when added *in vitro*, and 2) the delay in appearance of the sulfation factor in serum after the administration of growth hormone. In the rat this delay may be six to twelve hours, whereas in man it may be more than twenty-four hours. Sulfation factor may represent an activated "core" of growth hormone or it may be an entirely independent material.

The relationship between growth hormone and sulfation factor has a parallel in other experimental systems. Bornstein and Park (12) showed that injection of growth hormone and cortisone into hypophysectomized diabetic rats induced the formation of a serum component which inhibited the uptake of glucose by the rat diaphragm. The direct addition of the hormones to diaphragm did not produce a similar effect. A comparable serum component induced by growth hormone inhibits the transport of sugars in the perfused hearts of hypophysectomized rats (13).

The presence of undiminished sulfation factor activity in adult life appears paradoxical at first, but is not inconsistent with other parameters of growth hormone activity. The growth hormone content of the human pituitary does not change significantly with age (14). Assays of growth hormone-like material in human plasma by the tibial-line method (3) and the immunologic method (4) have also shown no clearly discernible relationship with age. Although the evidence is inconclusive, the suggestion is strong that the cessation of growth in man is not the result of waning growth hormone activity but the result of an alteration of end-organ potential.

We were prompted to examine the sulfation factor activity of serum from pregnant women by the finding of Contopoulos and Simpson (15) that serum from pregnant rats had enhanced growth hormone-like action (tibial-line assay). This growth-promoting effect of serum may be of placental rather than pituitary origin. The limited observations on pregnant women have failed to reveal a comparable change in serum sulfation factor activity.

Chromophobe adenomas and craniopharyngiomas are generally considered to be without hormonal secretion. If this be true, the level of serum sulfation factor provides a measure of the residual eosinophil function in the non-neoplastic portion of the gland. When compression of the normal

part of the gland has been virtually complete, low levels of sulfation factor would be expected. In the case of chromophobe tumors, it may not be correct to assume an absence of hormonal secretion. Morphologic characteristics are not always correlated with functional behavior. Patient H.Z. (Table 2B) had active acromegaly clinically with high levels of serum sulfation factor, yet secretory granules could not be recognized by either light or electron microscopy. Patient R.McC. (Table 2A) had the combination of persistent lactation, amenorrhea and pituitary tumor which is termed the Forbes-Albright syndrome (16). In cases of this syndrome in which the tumor has been examined histologically, secretory granules have not been prominent, although clinical evidence has indicated secretion of prolactin. Suggestive evidence of slight production of growth hormone in certain patients with chromophobe tumors was recognized by Harvey Cushing and termed "fugitive" acromegaly (17). These fragments of information have been cited as evidence that chromophobe tumors may secrete growth hormone and perhaps prolactin. The finding of normal quantities of serum sulfation factor in the presence of evidence of depressed thyrotropic, adrenotropic and gonadotropic secretion in patients with chromophobe tumor (J.W. and G.Z.) may be interpreted as indicating slight production of growth hormone by the tumor.

The clinical course of acromegaly is of great interest. Most patients exhibit a period of active growth which may persist for ten years or more, to be followed by a prolonged period during which skeletal findings are stationary, and some signs of hypopituitarism may supervene. This latter period has been loosely termed "burned out" acromegaly. The explanation of this phenomenon has never been completely clear. Compression of the uninvolved portions of the anterior pituitary gland will eventually lead to suppression of basophil function with probable decrease in the secretion of thyrotropic, adrenotropic and gonadotropic hormones. Production of growth hormone may be decreased because of changes in the functional character of the gland or necrosis of the bulk of the tumor. In our series, the sulfation factor level in the serum of most patients with acromegaly during the clinically inactive phase of the disease was normal, suggesting that overproduction of growth hormone had in fact subsided.

Acknowledgments

The investigations on N.H. and D.M. were carried out at the St. Louis Children's Hospital and were made possible by the generous cooperation and interest of Dr. John C. Herweg and Dr. Leonard T. Furlow.

The generosity of Dr. Olaf Pearson of New York and Dr. Maurice Raben of Boston in providing sera was most helpful. Dr. Raben's assistance in preparing human growth hormone is greatly appreciated.

We are indebted to Miss Marilyn Mitchell for painstaking technical assistance and to Dr. Eric Reiss for many valuable suggestions and continued interest in this project.

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METABOLIC CHANGES PRODUCED BY HUMAN GROWTH HORMONE (LI) IN A PITUITARY DWARF*

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ABSTRACT

The treatment of pituitary infantilism has been unsatisfactory in the past, since growth hormones of animal origin have been ineffective in the human. This failure of response has been attributed to differences in molecular weight and other physico-chemical characteristics between growth hormones of various species. In this study, human growth hormone, prepared by the method of Li from pituitaries obtained at autopsy, was administered to a female pituitary dwarf aged 11 years, 5 months. Metabolic balances were determined for an initial control period of seventeen days, followed by periods of five to ten days during which the hormone was given intramuscularly in doses of 5, 10, or 2.5 mg. daily. Nitrogen retention was demonstrated at all 3 dosage levels, but was most pronounced at the 5-mg. level. There was also some retention of sodium, potassium and phosphorus and a slight increase in the concentration of serum alkaline phosphatase. Calcium was excreted initially, but during long-term administration of the growth hormone calcium was retained. The fasting blood sugar level was unchanged and there was no glycosuria. The glucose tolerance curve was higher, but the effects seemed transient and were probably related to the dosage level. The only toxic effect noted was slight fluid retention, which occurred with the highest dosages and also seemed transient. These findings are in general agreement with those of workers using animal growth hormone and with those of other workers using human growth hormone. Whether or not human growth hormone is an effective anabolic agent in subjects with an intact pituitary is an important but unsolved question. Studies of long-term administration of this growth hormone with particular reference to its effect on height are in progress; initial observations indicate a satisfactory response of linear growth.

THE species difference, both in clinical response and in molecular structure, between various growth hormones has been well documented (1–4). Hormone prepared from the pituitaries of cattle and swine has

Received December 22, 1958.

* Aided by a grant from Ayerst Laboratories through Dr. John Jewell.

Presented in part (C.H.L.) before the Conference on Chemical Organization of Cells, Normal and Abnormal, at Madison, Wisconsin, August 23, 1958.

proved disappointing when used for stimulation of growth in human pituitary dwarfism (5), and only rarely has it produced any of the changes in body metabolism predicted from results observed in animals (6). However, hormone prepared from the pituitaries of monkeys has shown some of the anticipated metabolic effects in man (4, 7, 8), undoubtedly because of the primate origin and closer species relationship. Growth hormone extracted from the pituitaries of humans (2, 9) has been reported to have the expected metabolic effects in man (4, 8, 10).

In the present study, pituitary glands from human autopsy material were collected through the cooperation of several colleagues working in pathology, and growth hormone prepared from this source was administered to a patient with pituitary dwarfism.

The growth hormone[1] was prepared by a method described previously (2, 4). A highly purified substance was obtained which was shown to behave as a homogeneous protein by zone electrophoresis, ultracentrifugation and end-group analyses. Assay by the tibia test in hypophysectomized rats showed growth-promoting activity comparable to that of the bovine hormone (4). Also, the preparation seemed free from the other adenohypophyseal hormones, as shown by the conventional assay procedures for thyrotropin, prolactin, adrenocorticotropin and gonadotropins.

CASE REPORT

C.B. (U234941), an 11½-year-old girl had been seen at the University of California Medical Center in 1955 at the age of 8¾ years, with the complaint of slow growth. Inquiry into familial growth patterns revealed that the height of the mother was 5 feet 1 inch (155 cm.), and that of the father 5 feet 7 inches (170 cm.); both were the shortest members of their respective families. The growth of 4 younger female siblings was within normal channels.

The patient weighed 6 pounds 4 ounces (2841 Gm.) at birth and was the product of a normal first pregnancy and delivery. Of particular interest was an illness at the age of 6 months characterized by symptoms of respiratory infection and high fever which persisted for several weeks. Following this, some slowing of growth and of physical development was noted, and short stature definitely was apparent before the end of the first year. The growth rate remained very slow but general health was good. At the chronologic age of 5 years the bone age was estimated to be at the 2-year level.

No therapy had been given up to the time of the first hospital admission (March 25 to April 7, 1955), at the age of 8¾ years.

Examination at that time showed a small, well proportioned girl (Fig. 1A) whose height was 105 cm. (41½ inches) as compared to the normal average for her age of 132 cm. (52 inches). General physical and neurologic examinations showed nothing abnormal except for a wandering nystagmus, sluggish pupillary responses, and partial atrophy of the optic discs with defects in the lower temporal quadrants of both visual fields, greater on the right. Complete blood counts, urinalysis findings and serum electrolyte determi-

[1] Preparation of human growth hormone in the Hormone Research Laboratory was aided in part by grants from the American Cancer Society and the Lasker Foundation.

A B C

FIG. 1. *Patient C.B.* Pituitary dwarf.

A. March 29, 1955, aged 8 yrs. 8 mo., when first seen at U.C. Medical Center.

B. Jan. 3, 1958, aged 11 yrs. 6 mo., at the beginning of the present study.

C. Feb. 28, 1958, aged 11 yrs. 7 mo., at the height of fluid retention during teatment with 10 mg. of HGH daily.

nations were normal (Table 1). Thyroid function studies yielded the following results: serum level of protein-bound iodine 7.1 μg. per 100 ml., butanol-extractable iodine 4.0 μg. per 100 ml., thyroidal 24-hour I^{131} uptake 17.4 per cent, with a rise of 51.5 per cent after administration of 5 units of thyrotropin (Armour) every twelve hours for 4 doses. Urinary 17-ketosteroids were 0.5 mg., and 17-hydroxycorticoids 2.1 mg. per twenty-four hours. Bone age was $4\frac{1}{2}$ years at the chronologic age of $8\frac{3}{4}$ years. Skull roentgenograms were normal. An electroencephalogram was mildly abnormal, showing generalized changes including a moderate irregularity of form, a varying pattern of frequencies, and some sharp high-voltage waves; during hyperventilation there was occasional slowing to 2 to 3 per second. A pneumoencephalogram did not demonstrate any abnormalities. Psychometric tests revealed average intelligence (mental age, 8 years; I. Q., 92).

The diagnosis was pituitary dwarfism secondary to encephalitis at 6 months of age, with mild residual optic atrophy.

Because of the low borderline thyroidal I^{131} uptake and the good response to thyrotropic hormone, sodium *l*-thyroxine was started to see if the rate of growth could be increased. This was continued for two and a half years in an average dosage of 0.15 mg. daily, maintaining the serum protein-bound iodine level between 5.9 and 8.2 μg. per 100 ml. There was some initial loss of weight but no real stimulation of growth, which remained at a rate below that of the third percentile. There was acceleration of bone maturation, particularly in the first year of treatment (Fig. 2). Treatment was discontinued six weeks before the present study.

TABLE 1. CHEMICAL AND HORMONAL DETERMINATIONS ON BLOOD AND URINE

	Year 1955	Year 1958—Period of balance study				Long-term administration					
						Daily			Every other day		
		Control (Jan.)	5 mg. HGH daily	Control	2.5 mg. HGH daily	2.5 mg. Apr.	5 mg. May	5 mg. June	2.5 mg. July	2.5 mg. Aug.	5.0 mg. Sept.
Blood urea nitrogen (mg./100 ml.)		16.0	9.3	15.6	10.7	13.6	11.0	12.9	14.3	8.5	10.4
Fasting blood sugar (mg./100 ml.)		80.0	94.0	75.0	94.0	74.0	81.0	68.0	55.0	83.0	
Serum Na (mEq./L)	145.0	145.0	143.0	138.0	142.0	143.0	143.0		140.0	130.0	142.0
Serum K (mEq./L)	4.9	4.6	4.3	4.8	4.2	4.5	3.9	4.9	4.4	4.0	4.5
Serum CO_2 (mEq./L)		27.6	25.0	25.9	23.8	23.0					
Serum Cl (mEq./L)	100.0	103.0	102.0	106.0	105.0	103.0	108.0	109.0	103.0	103.0	103.0
Serum Ca (mg./100 ml.)	10.7	10.0	9.8	9.1	8.7	10.9	10.2	8.9	8.4	10.0	9.7
Serum P (mg./100 ml.)	5.2	4.7	5.2	5.4	5.4	5.2	5.4	5.5	5.3	5.1	5.5
Serum alk. phosphatase (Bodansky u.)	8.0	3.3		7.7	6.3	13.4	11.8	11.0	9.6	11.5	5.6
Serum cholesterol (mg./100 ml.)		248.0			190.0	268.0		206.0	333.0	178.0	246.0
Serum tot. protein (Gm. %)		6.5	6.7	6.9	6.4	8.3		7.2	7.2	7.2	7.3
Serum alb./glob. ratio		3.4/3.1	3.7/3.0	3.9/3.0	3.6/2.8	4.1/4.2		3.8/3.4	4.8/2.4	4.1/2.2	4.4/2.9
Serum PBI (μg./100 ml.)	7.1	7.2				6.8					
Urinary 17-OH-corticosteroids (mg./24 hrs.)	2.1	2.6		3.0	5.1	4.7			4.1	3.4	
		4.0							3.1	2.8	
Urinary 17-KS (mg./24 hrs.)	0.5	1.8		1.8	1.8	2.5			1.8	1.4	
		2.1							2.1	1.9	
Urinary gonadotropin (2.5 mouse units)		Neg. ×3			Borderline pos.	Neg.			Borderline pos. ×2		

For the study reported here, the patient was readmitted on December 30, 1957, remaining until March 4, 1958. Her age was now 11 years 5 months and her height was 113 cm. (44½ inches). This represented the average height for a girl of 5¾ years, the average height at age 11 years 5 months being 149 cm. (58¾ inches). She was well proportioned but slightly thin, weighing 18.4 Kg. (40½ pounds) (Fig. 1B). General physical examination yielded nothing remarkable. The eyes had improved; the left disc now appeared normal and the right showed only a slightly blurred margin and atrophic pattern. The visual field was normal on the left and there was a lessened temporal defect on the right.

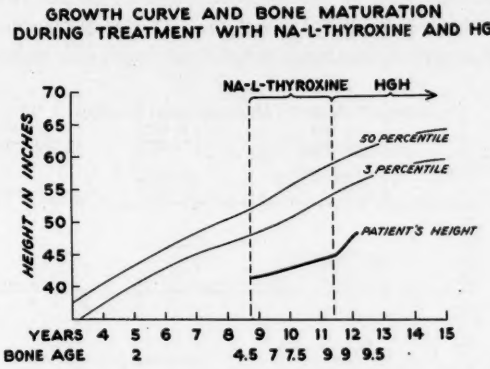

FIG. 2. During the treatment with sodium *l*-thyroxine, the rate of growth remained below that of the third percentile but bone age advanced from 4.5 to 9 years over a period of two and a half years.

Blood counts and urinalysis findings were normal. Reaction to the tuberculin skin test was negative. Thyroid function studies showed a serum protein-bound iodine level of 7.2 μg. per 100 ml.; a 24-hour thyroidal I^{131} uptake of 18 per cent with a rise to 25 per cent after 12 units of thyrotropin (Armour); and a BMR of −2 per cent. Bone age was now 9 years (chronologic age, 11½ years). Roentgenograms of the skull were again normal. Urinary 17-ketosteroids were 2.1 and 1.8 mg., and 17-hydroxycorticoids 4.0 and 2.6 mg. per twenty-four hours. The cells of a buccal smear showed nuclear sex chromatin (female). These and other results are tabulated in Table 1.

PLAN OF STUDY

Human growth hormone (HGH) was administered intramuscularly in physiologic sodium chloride solution, in which it is easily soluble. No other medication was given during the period of study. After an initial control period, the schedule of injections was as follows: 1) 5 mg. daily for ten days; 2) control period seven days; 3) 10 mg. daily for five days; 4) control period five days; 5) 2.5 mg. daily for ten days.

Metabolic balance studies were continuous during this time, with a fixed weighed dietary intake of 1600 calories containing 172 Gm. of carbohydrate, 70 Gm. of protein and 70 Gm. of fat, 50 mEq. of sodium, 70 mEq. of potassium, 1.16 Gm. of calcium and 1.33 Gm. of phosphorus. Exactly the same diet, specially prepared in the Metabolic Kitchen, was continued for the entire period of study. Refusal occurred on only one day, and appropriate subtraction from intake was made for that day.

All urine and stools were collected. Determinations were made on each 24-hour specimen of urine. Stool specimens were pooled for each experimental period (carmine-red marking). Routine methods, with some modifications for microdeterminations on serum, were used for all analyses. Urinary 17-hydroxycorticoids were determined by a modified Reddy extraction method and Porter-Silber colorimetry (11, 12), and 17-ketosteroids by the Callow-Zimmermann reaction following hydrolysis and ether extraction (13, 14).

RESULTS AND DISCUSSION

There were no reactions to the injections of HGH, although there was minimal soreness with the highest dosage. The patient's general health remained good. The patient gained weight during each period of hormone

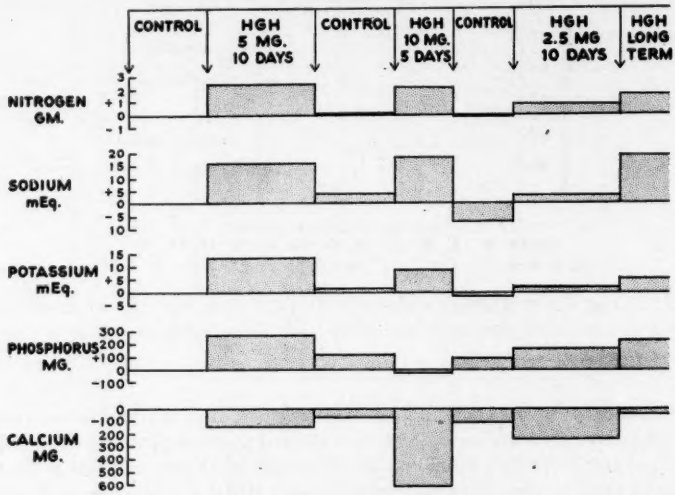

FIG. 3. Results of balance studies. Changes above the initial control baseline represent retention of the substance; below the line, loss. Long-term results in the right-hand column were obtained after daily administration of 2.5 mg. of HGH for two months.

administration and lost weight during each succeeding control period. These weight changes were most marked during the 10-mg. dosage, when puffiness of the face was distinctly apparent (Fig. 1C) and there was minimal pretibial pitting edema. This was transient, however, and did not recur during long-term treatment.

Chemical and hormonal data on the blood and urine are listed in Table 1. Results of balance studies are shown in Figure 3, with the zero levels representing the values during the initial control period of seventeen days. (At this time there were slightly positive balances for all substances measured.) In Figures 4 and 5 the values are adjusted to show daily urinary excretion of nitrogen and calcium per milligram of urinary creatinine.

The most striking effect of HGH therapy was a *decrease in urinary nitrogen*, indicating retention of this substance. This effect was apparent by the second day of each period of administration, and continued for one day when the hormone was stopped (Fig. 4). During the dosage of 10 mg. daily there was only slightly greater retention of nitrogen than during the dosage of 5 mg. daily. When the dosage was reduced to 2.5 mg. daily, there was still retention of nitrogen, but it was relatively slight. This seemed to indicate that in this patient 5 mg. per day approximated the optimal dosage for retention of nitrogen.

Associated with this retention of nitrogen was a drop in the level of blood

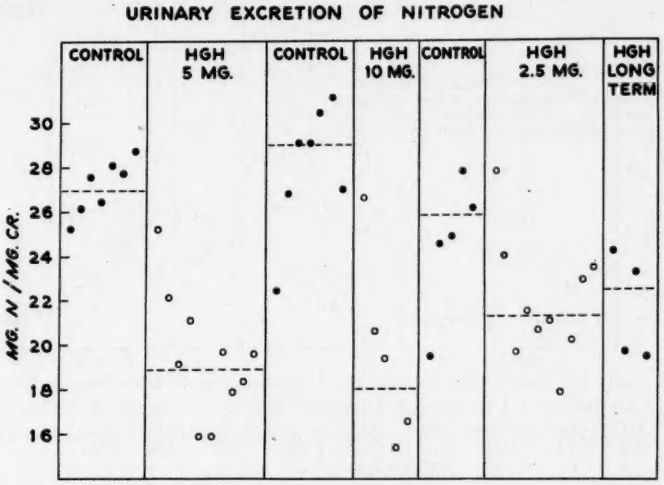

FIG. 4. Daily urinary excretion of nitrogen in terms of creatinine excretion. Lines represent average values for the period concerned. Note that the change in urinary nitrogen occurred within 48 hours.

urea nitrogen, with a return to control levels following cessation of hormone therapy (Table 1). Fecal nitrogen was slightly, but not significantly, lower during each experimental period. Total balance studies (Fig. 3) showed that nitrogen retention occurred at all dosage levels.

Studies of the effect of bovine growth hormone in man have not shown this immediate retention of nitrogen during the period of administration, although Shorr (who used very large doses) reported some nitrogen retention after a long period of administration (6).

Raben (9), who used human growth hormone prepared in a slightly different fashion, and other workers (8, 15–17) using his preparation, have demonstrated a similar nitrogen-retaining effect in patients with pituitary

insufficiency. Reports are conflicting concerning such a retention in subjects with an intact pituitary. Pearson observed no effect in a non-hypophysectomized patient (15), and Bergenstal noted no response to either the Li or the Raben preparation in normal children but obtained some effect in normal adults (16). Ikkos *et al.* recently reported a marked anabolic effect in normal adults from administration of a human growth hormone prepared by the method of Li (10). If effective anabolism occurs in the

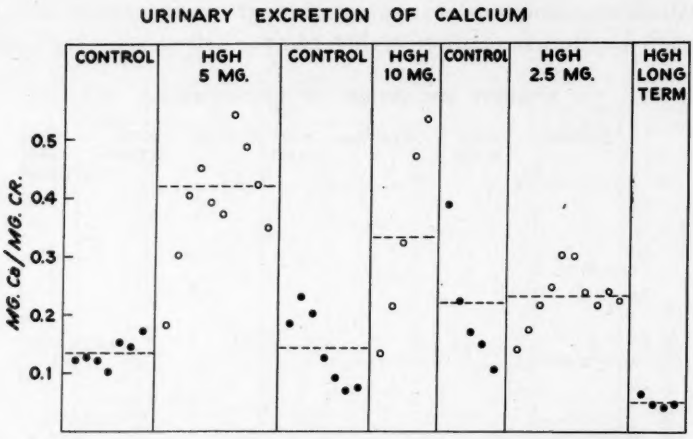

FIG. 5. Daily calcium excretion in terms of creatinine excretion. Lines represent average values for the period concerned. Calcium balance was negative during the initial periods of HGH therapy, but became positive during long-term administration.

normal subject, growth hormone would be useful in many clinical conditions, as well as in the hypopituitary state.

In our patient, sodium, potassium and phosphorus were also retained during the experimental periods (Fig. 3). The values returned promptly to the baseline levels during the control periods. Similar findings have been reported with the use of the Raben preparation (8, 17). The serum alkaline phosphatase level showed a slight rise, but no significant changes occurred in the serum concentration of inorganic phosphorus or the other electrolytes studied (Table 1). This finding is somewhat at variance with the results of Henneman *et al.* (17) and Raben and Hollenberg (18) using Raben's preparation of growth hormone, and with earlier animal work, since a progressive rise in the serum levels of alkaline phosphatase and inorganic phosphorus is reported.

The change in calcium metabolism was initially in the opposite direction from that of the other electrolytes (Figs. 3 and 5). Calcium excretion

increased markedly during the experimental periods, followed by a return to base-line values during control periods. However, later balance studies following continuous administration of 2.5 mg. of HGH daily for two months showed that the calcium balance had become positive. Henneman *et al.* (17) also reported an initial negative calcium balance followed later by retention. However, Beck *et al.* (8) demonstrated retention immediately after administration of the hormone.

Effects on glucose metabolism seemed important because of the previously reported diabetogenic effects of growth hormone in animals (19, 20).

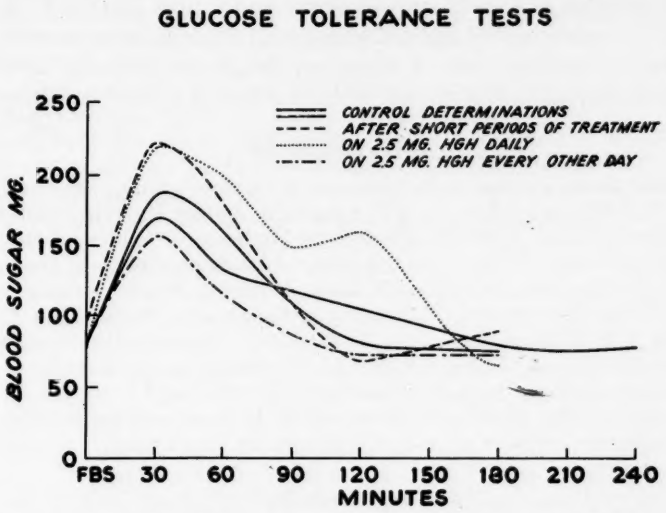

FIG. 6. Note the higher and more prolonged curves after treatment with HGH, although the most recent (lowest) one is below the control curves. (Blood sugar values, mg. per 100 ml.)

In our patient the fasting blood sugar level did not change appreciably (Table 1), but the glucose tolerance curves were higher during both the acute trials and the long-term administration (Fig. 6). The most recently determined blood sugar curve, obtained after treatment for two months with 2.5 mg. of HGH every other day, showed a return to below control levels. No glycosuria was observed, even at the highest dosage levels. This decreased glucose tolerance also was reported by Beck and co-workers (8) with the use of the Raben extract, but only a minimal effect was noted by Henneman *et al.* (17). Bergenstal and associates (21), who used the Li human growth hormone, noted a decrease in glucose tolerance and also some evidence of increased insulin resistance. Luft *et al.* (22) and Pearson

(15) reported a marked exaggeration of the diabetic state when human growth hormone was administered to hypophysectomized and to intact diabetics. The possibility that these changes in carbohydrate tolerance are caused by impurities in the hormone preparations has been suggested.

No significant changes were noted in the level of serum protein-bound iodine, or in urinary 17-ketosteroids, 17-hydroxycorticoids or gonadotropin (Table 1). The last two gonadotropin determinations listed on Table 1 were borderline-positive at 2.5 mouse units. Whether this is significant will be ascertained only with repeated studies during periods with and without HGH therapy.

Administration of human growth hormone for nine months to date has resulted in a growth of 9 cm. compared to a pretreatment growth of approximately 3 cm. per year. A report on long-term treatment of this patient, with adequate controls, will be the subject of a later communication.

Acknowledgments

Particular thanks are due to our colleagues in various pathology departments who collected the pituitaries for use in this study—Drs. Robert R. Wright and John W. Swift of the Herbert C. Moffitt Hospital of the University of California; Drs. Samuel W. French and Jesse L. Carr of the University of California Pathology Service at the San Francisco Hospital; Dr. Arthur R. Lack of the San Mateo Community Health Center; and Col. Nelson S. Irey, M.C., of the Letterman Army Hospital, San Francisco.

We also wish to thank Prof. Peter H. Forsham, Director of the Metabolic Unit, University of California Medical Center, for helpful advice and guidance.

This study would not have been possible without the help of the laboratory and dietary workers in the Metabolic Unit as well as the workers in the Pediatric Microchemical Laboratory of the University of California Medical Center.

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THE EFFECT OF BETAINE ON HUMAN SERUM LIPOPROTEINS*

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ABSTRACT

The effect of long-term administration of betacyamine, a mixture of betaine and guanidoacetic acid, on the concentration of serum lipoproteins was studied in 6 out-patients. A similar controlled study was made of the effect of betaine on the concentration of serum lipoproteins in 3 groups of institutionalized chronic psychiatric patients. A definite hyperlipoproteinemic and hypercholesterolemic effect of betaine was discovered, involving all low-density serum lipoproteins (except the $S_f^\circ 100–400$ class) and the major high-density serum lipoprotein class, HDL-3. The largest increases were observed in the $S_f^\circ 0–12$ class. The increased levels of low-density serum lipoprotein resulted in concomitant elevations in atherogenic index values. Female patients showed significantly greater elevations in the concentrations of $S_f^\circ 0–12$ and $S_f^\circ 12–20$ serum lipoproteins and total cholesterol than did male patients. The association of high atherogenic index values with an increasing incidence of morbidity and mortality from coronary artery disease contraindicates the use of hyperlipoproteinemic agents such as betaine in the treatment of patients with coronary artery disease. Biochemical mechanisms by which betaine may produce its hyperlipoproteinemic effect are discussed.

REPORTS that betaine lowers the level of total serum cholesterol (1) and that it is beneficial in the treatment of human coronary atherosclerosis in man led to the present study of the effect of betaine on the concentration of human serum lipoproteins.

CLINICAL MATERIAL AND METHODS

Out-patients (betacyamine)

Six patients (3 males and 3 females) were seen on an out-patient basis for periods ranging from eleven to thirty-six months, during which time from 30 to 60 blood specimens were obtained on each. Table 1 contains a summary of the clinical data. All blood

Received November 5, 1958.

* Supported by a grant-in-aid from International Minerals and Chemicals Corporation.

samples were analyzed for concentration of serum lipoproteins and total serum cholesterol by methods previously reported (2, 3).

The preparation administered was betacyamine, a mixture of betaine and guanidoacetic acid (glycocyamine). Blood samples were obtained before treatment, during the course of treatment, and following cessation of treatment. Betacyamine was administered orally in gradually increasing doses (increments of 5 Gm. for betaine and of 1 Gm. for glycocyamine), until a total daily maintenance dose of 25 Gm. of betaine and 5 Gm. of glycocyamine was reached.

Hospitalized patients (betaine)

The effect of betaine without glycocyamine was studied in 3 different groups of chronic ambulatory psychiatric patients hospitalized in 2 state mental institutions.

Group 1 consisted of 13 male and 3 female patients ranging in age from 25 to 63 years, hospitalized in institution A. The men received betaine daily for a period of one month, the women for sixteen weeks. All received a total daily dose of 15 Gm. of betaine administered in fruit juice in three fractional doses of 5 Gm. each. Blood samples were obtained after two and four weeks of this medication in the men, and after two, four, seven, ten and twelve weeks in the women. Weight and blood pressure were recorded each time. Three blood samples spaced one week apart were obtained on all patients before administration of betaine, and 2 samples were obtained at varying intervals following cessation of betaine therapy. Lipoprotein and serum cholesterol determinations were made on all blood specimens.

Group 2 consisted of 24 male patients ranging in age from 29 to 74 years, hospitalized in institution B. Of these, 5 (mean age 53 years) were studied concurrently as control subjects not receiving any medication. The remaining 19 (mean age 56 years) received 15 Gm. of betaine daily divided into two equal doses, for a period of twenty weeks. Blood samples were obtained serially during this 20-week period, as well as before and after treatment. Seventeen samples were obtained from each treated and control patient over a period of fourteen months. Thus the data on Group 2 are based on the analysis of 408 (17×24) blood specimens.

The results in Groups 1 and 2 suggested that female patients receiving betaine show a greater increase in the concentration of serum lipoproteins than do male patients. To test this impression, 17 female chronic psychiatric patients ranging in age from 24 to 60 years, hospitalized in institution A, were studied for a period of thirty-one weeks. They were designated Group 3, and included 7 controls (mean age 44 years) and 10 patients receiving betaine (mean age 44 years). The former received no medication; the latter ingested 15 Gm. of betaine daily in two equal doses, for a period of sixteen weeks. Nineteen blood samples were obtained from each patient—4 during the four-week pre-treatment period, 11 during the sixteen weeks of treatment, and 4 during the ten-week post-treatment period. Thus the data on Group 3 are based on the analysis of 323 (17×19) blood specimens.

RESULTS

In both the out-patient and in-patient studies it was found that administration of betaine produced an elevation in the concentration of certain of the low-density serum lipoproteins and of total serum cholesterol. Statistical analyses of the data showed that the elevation in the levels of $S_f^{\circ}0$–12 and $S_f^{\circ}12$–20 serum lipoproteins and of total serum cholesterol was

highly significant, that the elevation of the $S_f^\circ 20–100$ lipoprotein level was less consistent, and that there was no significant change in the level of $S_f^\circ 100–400$ lipoproteins.

Tables 1 and 2 contain summaries of the data (and their statistical significance) obtained in the out-patient and in-patient study, respectively. Figure 1 shows mean serum lipoprotein concentrations charted in terms of weeks of betaine therapy in the 10 treated female patients of Group 3. A detailed discussion of the changes observed in each band of serum lipoproteins follows.

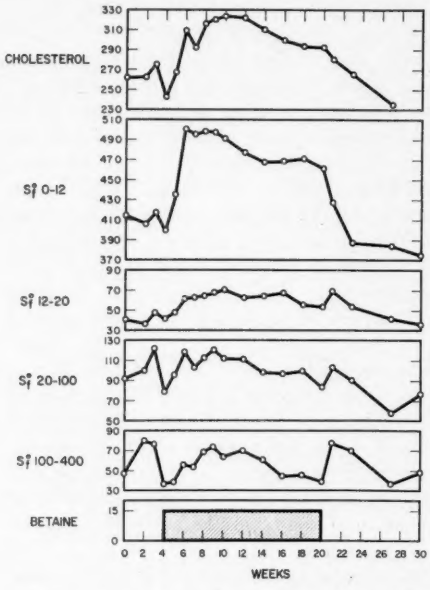

FIG. 1. Curves of the concentrations of serum lipoproteins and total cholesterol (mg. per 100 ml.) in relation to time of therapy (weeks) in 10 female patients receiving 15 Gm. of betaine daily.

Changes in $S_f^\circ 0–12$ serum lipoprotein levels

In the out-patient study with betacyamine, 3 of the 6 patients (B.H., M.F. and M.J.) showed pronounced elevations (significant at the 0.1 per cent level) in the concentration of $S_f^\circ 0–12$ lipoproteins, and 2 (G.R. and L.H.) showed somewhat smaller increases (significant at the 5 and 2 per cent level, respectively). It is of interest that the latter 2 patients initially had the abnormally elevated $S_f^\circ 20–100$ lipoprotein levels characteristic of xanthoma tuberosum (4, 5). No definite statement can be made about the $S_f^\circ 0–12$ values in the sixth patient (E.M.) because of the extremely elevated initial levels associated with xanthoma tendinosum (4, 5) which did not permit accurate partition between the $S_f^\circ 0–12$ and $S_f^\circ 12–20$ bands. Therefore the effect of betacyamine had to be evaluated on the combined bands of $S_f^\circ 0–20$, which showed a pronounced elevation, significant at the 0.1 per cent level. This finding suggests that an extremely high initial concentration of $S_f^\circ 0–12$ lipoproteins per se does not obviate the effect of betacyamine in raising the level of $S_f^\circ 0–20$ lipoproteins.

A statistically highly significant and fairly large increase in $S_f^\circ 0–12$ serum lipoprotein concentration was observed in all 3 groups of institutionalized patients (Table 2). Thus 15 of 16 treated patients in Group 1, 15 of 19

TABLE 1. SUMMARY OF RESULTS IN 6 OUT-PATIENTS TREATED WITH BETACYAMINE (BETAINE+GLYCOCYAMINE)

Patient, Sex, Age, Diagnosis	S_f^*0–12			S_f^*12–20			S_f^*20–100			S_f^*100–400			Atherogenic index			Cholesterol		
	No treat.*	Treat.**	Change† & signif.	No treat.	Treat.	Change & signif.	No treat.	Treat.	Change & signif.	No treat.	Treat.	Change & signif.	No treat.	Treat.	Change & signif.	No treat.	Treat.	Change & signif.
B.H.—M55 Myocard. infarct (old)	350	425	+75 P <0.001	41	60	+19 P <0.001	70	78	+ 8 N.S.‡	22	22	0 N.S.	58	71	+13 P <0.001	227	266	+ 39 P <0.001
M.F.—F60 Hypertension, CVA	353	439	+86 P <0.001	43	60	+17 P <0.01	109	115	+ 6 N.S.	63	53	− 10 N.S.	73	84	+11 P <0.02	234	265	+ 31 P <0.01
M.J.—M64 Hypertension, CVA	460	540	+80 P <0.001	41	72	+31 P <0.001	74	112	+ 38 P <0.001	27	59	+ 32 P <0.001	71	97	+26 P <0.001	255	309	+ 54 P <0.001
G.R.—M48 Xanthoma tuberosum	109	148	+39 P <0.05	80	114	+34 P <0.02	561	628	+ 67 N.S.	826	572	−254 P <0.01	267	245	−22 N.S.	593	484	−109 P <0.01
L.H.—F47 Xanthoma tuberosum	151	178	+27 P <0.02	118	133	+15 N.S.	356	368	+ 12 N.S	47	73	+ 26 N.S.	106	118	+12 N.S.	270	278	+ 8 N.S.
E.M.—F52 Xanthoma tendinosum	1165	1649	+484 P <0.001	=S_f^*0–20			29	166	+137 P <0.01	0	14	+ 14 N S	142	221	+79 P <0.001	651	783	+132 P <0.001

* The mean of all pre- and post-treatment values.

** The mean of all values obtained during treatment with betacyamine.

† Change is the difference between the mean of the values during treatment and the mean of the values before and after treatment.

‡ Not significant.

Note: S_f^*0–12, S_f^*12–20, S_f^*20–100 and S_f^*100–400 lipoprotein and cholesterol values are expressed in mg./100 ml. of serum; atherogenic index values are expressed in units.

TABLE 2. SUMMARY OF RESULTS IN 3 GROUPS OF HOSPITALIZED PATIENTS TREATED WITH BETAINE

	S_f^*0-12		S_f^*12-20		S_f^*20-100		S_f^*100-400		Cholesterol		Atherogenic index		HDL-1		HDL-2		HDL-3		Body weight	
	No treat.*	Treat.**	No treat.	Treat.	No treat.	Treat.	No treat.	Treat.	No treat.	Treat.	No treat.	Treat.	No treat.	Treat.	No treat.	Treat.	No treat.	Treat.	No treat.	Treat.
Group 1 (hosp. A) 13 males, 4 wks.	331	367 +36† P~0.02‡	38	46 + 8 P<0.01	79	110 +31 P<0.02	39	65 +26 N.S.§	213	238 +25 P<0.001	60	76 +16 P<0.01	25	35 +10 N.S.	41	50 +9 P<0.10	203	241 +38 P<0.001	141.8	141.9 +0.1 N.S.§
3 females, 16 wks.	371	500 +129	43	78 +35	63	74 +11	13	20 + 7	207	312 +105	58	80 +22	23	35 +12	85	89 +4	213	245 +32	100.3	101.2 +0.9
Group 2 (hosp. B) 19 males, 20 wks.	336	366 + 30 P<0.01	41	45 + 4 P<0.05	83	104 +21 P<0.01	38	47 + 9 N.S.	215	237 +22 P<0.001			25	28 + 3 N.S.	73	83 +10 P<0.01	144	210 +66 P<0.01	153.3	151.7 −1.6 N.S.
5 male controls	409	406 − 3	52	47 − 5	103	106 + 3	46	52 + 6	242	246 + 4			27	23 − 4	49	50 +1	179	188 + 9	153.9	153.3 −0.6
Group 3 (hosp. A) 10 females, 16 wks.	404	480 +76 P~0.001	48	62 +14 P<0.01	95	107 +12 P~0.05	64	57 − 7 N.S.	265	306 +41 P<0.001			28	37 + 9 P<0.001	98	89 − 9 P<0.01	227	251 +24 P<0.01	136.8	137.0 +0.2 N.S.
7 female controls	352	358 + 6	44	45 + 1	61	64 + 3	18	14 − 4	233	243 +10			25	27 + 2	110	110 0	236	250 +14	126.8	127.1 +0.3

* The mean of all pre- and post-treatment values.

** The mean of all values obtained during treatment with betaine.

† Change is the difference between the mean of the values during treatment and the mean of the values before and after treatment.

‡ Significance of the change.

§ Not significant.

Note: All low and high density serum lipoprotein and cholesterol values are expressed in mg./100 ml. of serum; atherogenic index values are expressed in units; and body weight in pounds

TABLE 3. SUMMARY OF DATA OBTAINED IN INDIVIDUAL PATIENTS IN GROUP 2 (19 MALES RECEIVING BETAINE FOR 20 WEEKS)

Patient	S_f^*0–12			S_f^*12–20	S_f^*20–100			S_f^*100–400	Cholesterol		
	No treatment*	Δ**	Signif. of Δ	Δ**	No treatment*	Δ**	Signif. of Δ	Δ**	No treatment*	Δ**	Signif. of Δ
B.A.	326	16	N.S.†	− 9	81	4	N.S.	4	203	−2	N.S.
C.A.	345	55	P<0.01	3	76	10	N.S.	− 3	226	24	N.S.
C.H.	326	38	P<0.05	1	94	19	P<0.05	− 5	230	29	P<0.01
D.E.	300	−77	P∼0.02	13	185	94	P<0.01	112‡	215	8	N.S.
E.S.	297	−11	N.S.	− 3	63	15	N.S.	− 1	204	2	N.S.
F.R.	365	77	P<0.01	28‡	103	29	P<0.02	−15	214	47	P<0.01
F.U.	378	74	P<0.02	29‡	93	42	P∼0.02	38‡	230	54	P<0.01
J.O.	336	4	N.S.	0	100	8	N.S.	−31	199	6	N.S.
J.O.S.	382	− 4	N.S.	− 7	88	15	N.S.	11	235	8	N.S.
K.R.	304	0	N.S.	− 2	81	7	N.S.	2	225	−4	N.S.
M.E.	226	59	P∼0.01	−10	70	21	N.S.	26	162	31	P<0.02
P.A.	308	19	N.S.	− 8	82	26	P<0.05	10	212	17	N.S.
P.I.	512	73	P<0.01	−11	64	3	N.S.	− 1	328	33	P∼0.02
S.C.	332	51	P<0.02	11	85	12	N.S.	8	205	34	P<0.01
S.H.	401	50	P<0.01	12	70	5	N.S.	−17	218	25	P∼0.02
T.I.	330	56	N.S.	21‡	98	48	P<0.01	27	203	26	N.S.
W.A.	363	85	P<0.05	− 1	67	19	N.S.	0	225	55	P<0.01
W.I.	230	2	N.S.	4	58	19	N.S.	− 1	164	8	N.S.
W.I.L.	332	6	N.S.	− 2	24	− 5	N.S.	0	194	9	N.S.

* The mean of all pre- and post-treatment values (7–8 samples per patient).

** Difference between the mean of the values during treatment (8 samples per patient) and the mean of the values before and after treatment.

† Not significant.

‡ Statistically significant changes (P=0.05 to P=0.01).

Note: S_f^*0–12, S_f^*12–20, S_f^*20–100 and S_f^*100–400 lipoprotein and cholesterol values are expressed in mg./100 ml. of serum.

treated patients in Group 2, and all 10 treated patients in Group 3 showed increases in the serum level of this class of lipoproteins.

In Table 3 the individual changes in the 19 treated patients of Group 2 are listed. It may be seen that in 9 of the 19 there were statistically significant increases in the $S_f^0$0–12 lipoprotein level and in only 1 was there a significant *decrease*. This patient (D.E.) initially had abnormally elevated levels of S_f^*20–100 and S_f^*100–400 serum lipoproteins. It is of interest that in the only other patient (Group 1) with a decrease in $S_f^0$0–12 lipoprotein concentration there was also a marked elevation in the initial levels of $S_f^0$20–100 and S_f^*100–400 lipoproteins. Lack of sufficient information concerning factors regulating individual serum lipoprotein classes in individual subjects prevents interpretation of the peculiar reaction to betaine in these 2 patients.

Changes in S_f^*12–20 lipoprotein levels

In the out-patient group of 6 subjects, the pattern of the $S_f^0$12–20 serum lipoproteins reflected quite closely that of the $S_f^0$0–12 lipoproteins. Thus the smaller elevations of $S_f^0$12–20 occurred in the 2 patients with xanthoma

tuberosum; in G.R. they were significant at the 2 per cent level, and in L.H. they were not statistically significant. Three of the remaining 4 patients (B.H., M.F. and M.J.) showed marked elevations in $S_f^{\circ}12–20$ concentration, significant at the 0.1, 1 and 0.1 per cent level, respectively. The high level of $S_f^{\circ}0–20$ lipoproteins in the patient with xanthoma tendinosum (E.M.) has already been mentioned.

In the hospitalized patients, there was a small though statistically significant increase in the mean level of $S_f^{\circ}12–20$ lipoproteins, with the women (Group 3) showing by far the largest mean increase (Table 2). In Table 3 it may be seen that for the individual male patients of Group 2 there were about equal numbers of plus and minus signs for the $S_f^{\circ}12–20$ value, though for 3 men (F.R., F.U. and F.L.) there was a statistically significant increase in Δ values, and no significantly negative Δ values were noted. In 12 of 13 male patients in Group 1 there were positive Δ values for $S_f^{\circ}12–20$; the single negative Δ value was not significant.

Changes in $S_f^{\circ}20–100$ lipoprotein levels

Only 2 of the 6 out-patients showed significant elevations in the serum concentration of $S_f^{\circ}20–100$ lipoproteins, namely, Patient E.M. with xanthoma tendinosum, and Patient M.J. with hypertension and a history of a cerebrovascular accident. No significant changes could be demonstrated in the remaining 4 subjects.

Among the institutionalized patients, statistically significant increases (about 20–30 mg. per 100 ml.) were observed in the mean $S_f^{\circ}20–100$ levels in the 2 male groups but not in the female group. Inspection of the individual responses of patients in Group 2 (Table 3) shows that 18 of 19 patients had positive Δ values for $S_f^{\circ}20–100$ lipoproteins, 6 of which were statistically significant.

Changes in $S_f^{\circ}100–400$ serum lipoprotein levels

Only 2 of the 6 out-patients showed significant changes in this lipoprotein band —— Patient M.J. in whom there was an increase significant at the 0.1 per cent level, and Patient G.R. with xanthoma tuberosum, in whom there was a decrease. This was the only decrease in any serum lipoprotein band observed in the out-patient group.

No significant changes in the concentration of $S_f^{\circ}100–400$ lipoproteins occurred in the 3 groups of hospitalized patients. In only 2 (D.E., Group 2; E.F., Group 1) of a total of 45 betaine-treated patients in Groups 1–3 were large increases noted in the values for this class of lipoproteins. It is of interest that these 2 patients initially had high levels of $S_f^{\circ}20–100$ lipoproteins and were also unique in responding to betaine with a *decrease* in the concentration of $S_f^{\circ}0–12$ lipoproteins.

Inspection of the individual responses of the patients in Group 2 (Table 3) shows a fairly even distribution of plus and minus signs, and only 2 significant Δ values for $S_f^o100–400$ lipoproteins, both positive.

Changes in the atherogenic index (A.I.) and in total serum cholesterol levels

In 4 of the 6 out-patients, A.I. values were significantly elevated, but remained unchanged in the 2 patients with xanthoma tuberosum. The level of total serum cholesterol rose markedly in the same 4 patients; however, in the 2 patients with xanthoma tuberosum, it remained unchanged in one (L.H.) and was only moderately elevated in the other (G.R.).

In the 3 groups of hospitalized patients, marked elevations in A.I. values and in serum cholesterol levels were observed, significant at the 1 and 0.1 per cent level, respectively.

Changes in body weight

Body weight remained fairly stable in the 3 groups of hospitalized patients. An effect of betaine on body weight is clearly excluded by the weight data summarized in Table 2.

DISCUSSION

The data show that betaine has a definite effect on serum lipoprotein concentrations, producing increases in all except the $S_f^o100–400$ low-density classes. The largest and most consistent elevations occurred in the $S_f^o0–12$ class. Statistically significant increases were also observed in the HDL-3 class, the major high-density lipoprotein component. As expected, along with these increases in serum lipoprotein concentrations, there were distinct elevations in the level of total serum cholesterol and in the atherogenic index.

Some quantitative differences in the response to betaine were noted between the male and female hospitalized patients. These differences are summarized in Table 4. It may be seen that there were significantly greater increases in the levels of $S_f^o0–12$ and $S_f^o12–20$ lipoproteins and total cholesterol in the female group. The levels of $S_f^o20–100$ and $S_f^o100–400$ lipoproteins were increased less in the female than in the male patients on the average, but these differences were not statistically significant. The reason for this sex difference in response to betaine is unknown.

Analysis of all the data failed to demonstrate any relationship between the initial level of serum lipoprotein and the subsequent change produced by betaine.

That betaine rather than glycocyamine is the effective agent in betacyamine is shown by 1) the results of the in-patient study in which only betaine was used, and 2) the fact that in 3 (G.R., L.H. and M.J.) of the 6

TABLE 4. DIFFERENCES BETWEEN MALE AND FEMALE PATIENTS IN THE RESPONSE TO BETAINE

	S_f^*0–12			S_f^*12–20			S_f^*20–100			S_f^*100–400			Cholesterol			Atherogenic index		
	No treat.*	Treat.**	Δ†	No treat.	Treat.	Δ	No treat.	Treat.	Δ	No treat.	Treat.	Δ	No treat.	Treat.	Δ	No treat.	Treat.	Δ
13 female patients‡ (16 wks.)	401	486	+85	43	66	+23	93	100	+ 7	53	49	− 4	250	309	+59	73	86	+13
13 male patients (4 wks.)	331	367	+36	38	46	+ 8	79	110	+31	39	65	+26	213	238	+25	60	75	+15
Difference between values (F-M)			49			15			−24			−30			+34			− 2
Significance of difference in Δ values			P <0.05			P <0.05			P <0.10			N.S.§			P <0.02			N.S.§

* Mean of pre-betaine values.
** Mean of values during treatment with betaine.
† Difference between the mean of the values during treatment and the mean of the values before treatment.
‡ The 10 female patients of Group 3 and the 3 female patients of Group 1.
§ Not significant.
Note: S_f^*0–12, S_f^*12–10, S_f^*20–100 and S_f^*100–400 lipoprotein and cholesterol values are expressed in mg./100 ml. of serum; atherogenic index values are expressed in units.

out-patients, betaine produced the same effect as did betacyamine. Our findings are in distinct contrast to those of Morrison (1), who reported that betaine produced a marked lowering of total serum cholesterol concentration in a group of patients with coronary atherosclerosis. However, direct and detailed comparison of the 2 studies is difficult because Morrison's patients were maintained with a low-fat, low-cholesterol diet and also had received previous treatment with choline and inositol.

The practical clinical application of our findings derives directly from the statistical relationship of the atherogenic index to mortality and morbidity from coronary artery disease (6, 7). The definite increase in the atherogenic index produced by betaine implicates betaine as a potentially dangerous substance for use in the treatment of patients with coronary artery disease. Furthermore, it would seem wise to reconsider the free and indiscriminate use of other "lipotropic" agents (*e.g.*, choline) until their effects on serum lipoprotein levels are known.

The mechanism by which betaine increases the concentration of serum $S_f^{\circ}0$–20 lipoproteins and total cholesterol is by no means clear. The biochemical pathways of betaine metabolism are known in some detail and have been reviewed by Mackenzie (8). The known metabolic products of betaine are n-dimethylglycine, n-methylglycine, glycine, an active one-carbon intermediate of the oxidation state of formaldehyde plus small and variable quantities of formaldehyde, and serine formed by condensation of glycine with the one-carbon intermediate. Betaine is also a methyl donor in the transmethylation reaction forming methionine from homocysteine. In the absence of clinical evidence for the accumulation of formaldehyde, the only potentially toxic and unphysiologic compound in the foregoing sequence, it appears unlikely that the effect of betaine on serum lipoproteins is mediated via any of the remaining metabolites along the known metabolic pathways of betaine. The role of choline as a lipotropic agent has been reviewed in detail (9). It seems unlikely that the effect of betaine on serum lipoproteins can be explained on the basis of "lipotropic action" in patients who maintain their weight while receiving a diet presumably adequate in protein and lipotropic agents. Mobilization of depot fat by betaine or choline also does not offer an adequate explanation, for the same reasons. It has been shown that choline accelerates synthesis of hepatic phospholipid (10) but the possible relationship of this finding to an increase in the concentration of low-density serum lipoproteins is not clear, even though a relationship between *serum* phospholipid and serum cholesterol levels has been suggested recently (11).

Desiccated thyroid (12–14), thyroxine (15) and triiodothyronine (16) each cause a marked lowering of the $S_f^{\circ}0$–20 serum lipoprotein level, and vitamin B_{12} has been shown to prevent weight loss and sometimes death

due to thyrotoxicosis in young rats (17–19); the suggestion has also been made that thyrotoxicosis is primarily a deficiency of vitamin B_{12} (20). A vitamin B_{12} deficiency has been postulated to explain the decreased rate of methylation of nicotinamide observed in hyperthyroid rats (21), and the requirement of vitamin B_{12} for the synthesis of methyl groups is well known, though transmethylation reactions probably do not require this vitamin (22, 23).

Furthermore, in rats, a deficiency of dietary betaine produced by ingestion of ethanolamine may be compensated for by an increase in methyl synthesis following administration of vitamin B_{12} (24). Low plasma B_{12} levels have been reported in strict vegetarians (25), and low serum cholesterol and S_f0–20 lipoprotein levels have been repeatedly observed in human subjects and animals receiving low animal-fat or vegetarian diets (26–29). These diverse observations are consistent with the hypothesis that low levels of vitamin B_{12} are in some way related to low levels of S_f0–20 serum lipoproteins, and high levels of vitamin B_{12} to high levels of S_f0–20 serum lipoproteins and cholesterol.

An effect of betaine on the absorption of fats or of some nutrient involved in the regulation of fat metabolism represents another mechanism by which betaine might cause the observed changes in serum lipoprotein concentration. However, the absence of a significant weight loss makes a defect in fat absorption an unlikely explanation.

Acknowledgment

We gratefully acknowledge the assistance of the ward personnel of the Napa and Stockton State Hospitals; of W. A. Oliver, M.D., Chief of Medical Services, Napa State Hospital; of Elmer F. Galioni, M.D., Chief of Medical Services, and Mrs. Hattie Green, Stockton State Hospital; and of the technical personnel of the Ultracentrifugal Group at the Donner Laboratory.

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JCEM

EFFECTS OF ESTROGEN AND TESTOSTERONE ON CIRCULATING THYROID HORMONE*

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ABSTRACT

A study was made to determine the effects of estrogen and androgen on circulating thyroid hormone. Administration of estrogen to athyrotic patients receiving adequate replacement therapy resulted in an increase in the level of serum protein-bound iodine (PBI), indicating that a functioning thyroid gland is not necessary for this effect. The increase in PBI concentration was greater in patients treated with thyroxine than in those treated with desiccated thyroid. Estrogen caused no elevation of the PBI level in hypothyroid patients receiving inadequate replacement therapy in whom the thyroxine-binding capacity of the serum was already increased. Estrogen also caused a prolongation of the thyroxine survival time (from 6.9 to 10.6 days, average), indicating a decreased rate of peripheral disposal of thyroxine.

According to current information, the administration of estrogen results in: 1) an increase in the thyroxine-binding capacity of serum, 2) an increase in the concentration of PBI, 3) a decrease in the utilization of thyroid hormone, 4) a decrease in the basal metabolic rate, 5) an increase in the pituitary secretion of thyrotropin, and 6) an increase in thyroidal activity. Neither the thyroid nor the pituitary gland is essential for the first four steps, but each plays a role in the intact organism. The effect of testosterone therapy on the thyroid hormone was indicated by a consistent fall in the serum PBI level, but the mechanisms involved in this change were not clearly defined.

ELEVATION of the serum protein-bound iodine (PBI) level during pregnancy was first reported by Heinemann, Johnson and Man in 1948 (1). This elevation appeared to be a physiologic response to pregnancy and was not accompanied by signs or symptoms of hyperthyroidism. Engstrom, Markardt and Liebman (2) in 1952 reported that a rise in the level of PBI could be induced in normal nonpregnant subjects by administration of estrogen. Investigations by Dowling, Freinkel and Ingbar demonstrated an increased ability of serum proteins, specifically inter-alpha globulin, to bind thyroxine during pregnancy (3) and during administration of estrogen (4). This fraction has been called the thyroxine-

Received December 12, 1958.

* Supported in part by United States Public Health Service Grants A-1200 and 2A 5023.

binding protein (TBP) or thyroxine-binding globulin (TBG). Since an increase in the total quantity of circulating thyroid hormone must be accomplished by increased rate of production or by a decreased rate of disposal of hormone, or both, further studies seem indicated in order to clarify the mechanism of the estrogen-induced rise in serum PBI concentration.

As a counterpart to the investigations involving the effects of estrogen on thyroid hormone, work has also been carried out concerning the effects of testosterone. Keitel and Sherer (5) demonstrated a definite fall in the serum PBI level with administration of testosterone to various patients; Federman, Robbins and Rall (6) confirmed this finding and suggested the mechanism of action of testosterone. The present report deals with the effects of estrogen and testosterone on serum PBI concentration, thyroxine-binding and rate of disposal of thyroxine, in an attempt further to elucidate the actions of these sex hormones on the circulating thyroid hormone.

MATERIAL AND METHODS

Estrogen was administered orally as diethylstilbestrol, 10 mg. three times daily, to 14 athyrotic patients. Of these, 1 received no replacement therapy; 10 received desiccated thyroid in various dosage; and 3 received thyroxine. Serum PBI determinations (by the digestion-distillation method (7, 8)) were made before, during and after the period of estrogen administration. Thyroxine-binding capacity was determined on serum from 6 patients before and during estrogen therapy.

Thyroxine-survival studies were performed on 17 male and 3 female adults, employing the technic described by Sterling, Lashof and Man (9). The patients were given 50 to 80 microcuries of I^{131}-labeled sodium *l*-thyroxine (Abbott) intravenously. Recirculation of radioactive iodine was minimized by administration of 5 drops of saturated solution of potassium iodide three times daily throughout the period of study. Blood was drawn fifteen minutes after the injection of thyroxine, and daily thereafter. A 3-ml. aliquot of serum from each sample was counted in a well scintillation counter and the results expressed as a percentage of the initial (fifteen-minute) sample. Results were plotted against time on semi-log paper. After a period of six to ten days during which the control curve was established, diethylstilbestrol was administered orally in a dosage of 10 mg. three times daily to 15 subjects in order to determine the effect of estrogen on the survival of thyroxine. In another group of 5 subjects, testosterone propionate was administered intramuscularly in a dosage of 50 mg. daily, also to test its possible effect on thyroxine half-life.

The thyroxine-binding capacity of serum was determined by the semi-quantitative method of Dowling *et al.* (3). A minor deviation from their procedure was the substitution of bovine for human serum albumin in the various solutions. Separation of proteins were carried out in a Spinco paper electrophoresis apparatus. These determinations were performed on serum from hypothyroid patients before and during administration of estrogen or testosterone.

Nine euthyroid adult males were studied to determine the effect of testosterone on serum PBI concentration, thyroxine-binding capacity and serum protein fractions. After control periods, these men were given 50 mg. of testosterone propionate intramuscularly daily for periods of fourteen to twenty days. Protein fractions were de-

termined by the Spinco Analytrol method on the paper electrophoretic strip. In another group of 13 adults, only the PBI level was determined before and during administration of testosterone. Of these 13 subjects, 8 received testosterone propionate intramuscularly in a dosage of 25 to 50 mg. daily, and 5 received methyltestosterone orally in a dosage of 50 to 60 mg. daily.

RESULTS

Administration of estrogen to an untreated myxedematous patient caused no increase in the serum PBI level. In hypothyroid subjects receiving inadequate replacement therapy with desiccated thyroid there was either no rise or only a slight rise in the PBI level; in those maintained in a completely euthyroid state, a definite rise was induced. Marked increases were noted in the 3 patients maintained on thyroxine; in these, the PBI level rose from 7.6, 8.5 and 9.1 μg. per 100 ml. to 12.0, 13.9 and 22.3 μg. per 100 ml., respectively (Fig. 1).

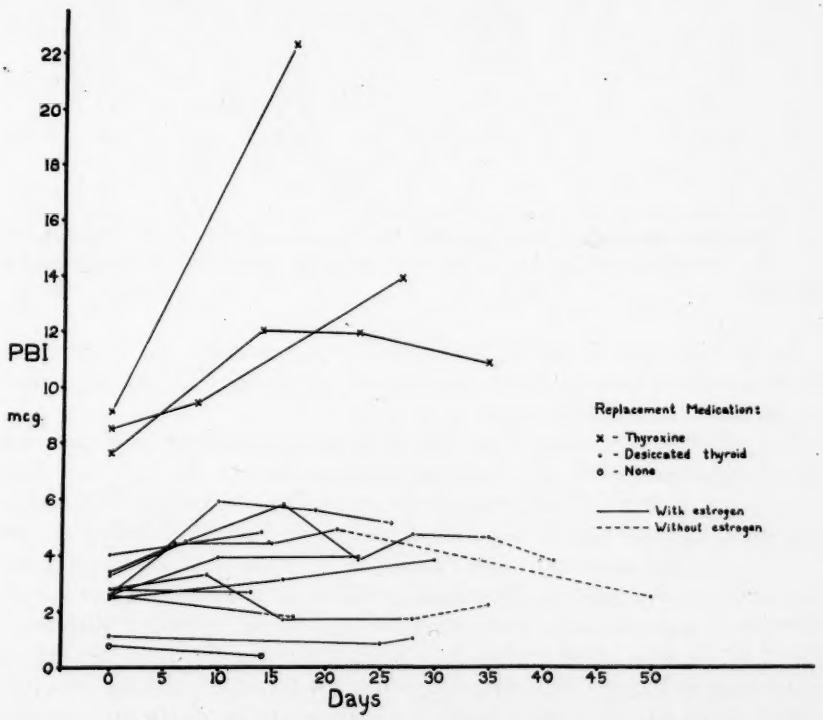

FIG. 1. Effect of administration of diethylstilbestrol, 10 mg. three times daily, on the serum PBI level (μg./100 ml.) in myxedematous patients treated with variable amounts of thyroxine or desiccated thyroid.

TABLE 1. SUMMARY OF DATA ON 15 SUBJECTS IN WHOM THE EFFECT OF ESTROGEN ON THYROXINE SURVIVAL WAS STUDIED

(Estrogen was administered orally as diethylstilbestrol, 10 mg. three times daily)

Subject		Thyroxine half-life (days)	
Name	Sex & Age	Before estrogen	With estrogen (max.)
V.G.*	M 56	5.6	8.6
C.T.	M 86	8.0	12.2
W.C.	M 73	7.4	10.0
F.J.	M 75	6.2	12.4
W.G.	M 78	7.8	12.2
P.R.	M 77	7.2	8.6
J.B.	M 68	5.5	8.8
G.N.	M 82	8.2	12.8
W.H.	M 80	5.6	8.8
J.P.	M 80	7.0	12.4
E.P.	F 58	7.4	10.4
T.B.	M 66	5.0	8.8
O.G.	F 54	8.4	11.6
C.M.	M 37	7.5	11.4
H.S.†	M 45	6.3	6.3
		Avge. 6.9	*10.6*

* Myxedematous patient receiving adequate replacement therapy with thyroxine.

† Not included in average. Myxedematous patient receiving inadequate replacement therapy.

In the 6 patients in whom thyroxine-binding capacity was determined, binding was increased in every instance during estrogen therapy, regardless of the initial serum PBI level.

The effect of administration of estrogen on thyroxine survival time was clearly apparent in the 13 euthyroid subjects studied. In each case there was a prompt change in the slope of the curve, the average half-life increasing from 6.9 days during the control period, to 10.6 days during the period of estrogen administration (Table 1). In 6 subjects the slope of the survival curve changed as illustrated in Figure 2. However, in 7 of the 13, there was a particularly pronounced retention of thyroxine within the blood in the first three to five days after beginning estrogen therapy, as illustrated in Figure 3. Because this effect was transient, the survival time was determined from the relatively rectilinear portion of the curve during the succeeding days of the study. In 1 hypothyroid patient (H.S.) receiving inadequate replacement therapy, there was no alteration of the thyroxine survival curve. In another hypothyroid patient (V.G.) receiving

adequate replacement therapy with thyroxine, the half-life changed from 5.6 to 8.6 days during administration of estrogen.

In 4 of 5 subjects, administration of testosterone propionate caused no apparent change in the slope of the thyroxine survival curve; in the remaining case, the half-life dropped from 6.9 to 5.6 days (Table 2).

The thyroxine-binding capacity of serum from untreated myxedematous patients was increased almost to the degree noted in pregnancy or in estrogen-treated subjects (Fig. 4). The increased binding capacity in hypo-

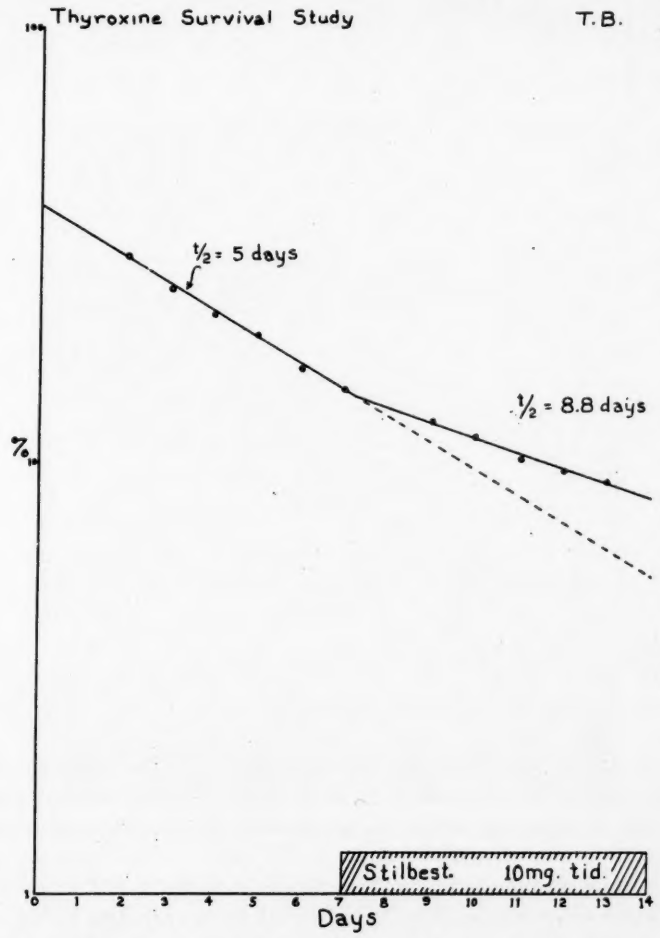

FIG. 2. Effect of administration of estrogen on thyroxine survival time (half-life) in a euthyroid subject.

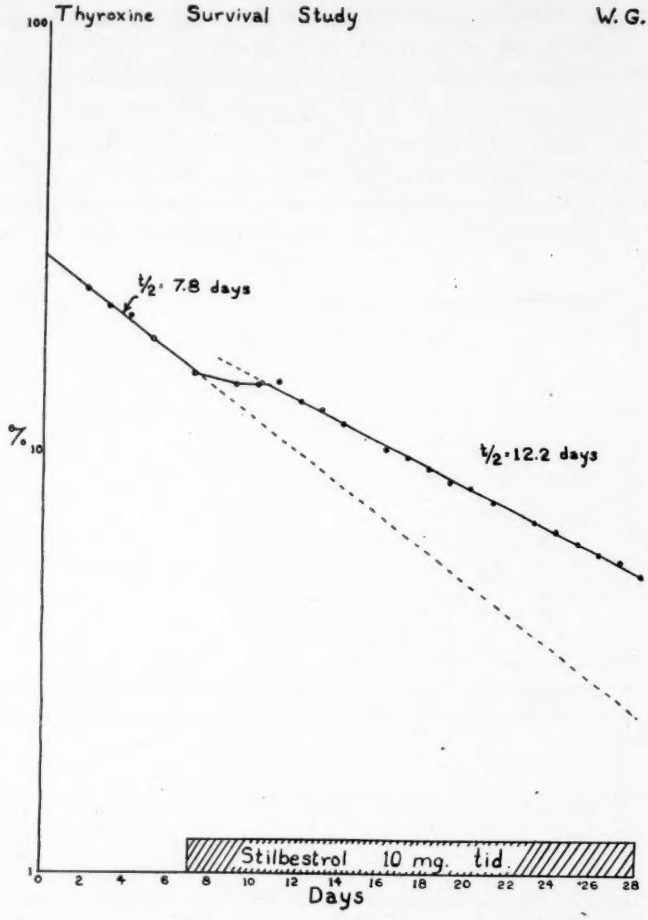

FIG. 3. Effect of administration of estrogen on thyroxine survival time (half-life) in a euthyroid subject. The more pronounced retention of thyroxine upon initiation of estrogen therapy was noted in 7 of 13 subjects studied.

thyroidism has been reported by Albright *et al.* (10). In 1 untreated myxedematous patient, the binding capacity was increased further upon administration of estrogen while the serum PBI value remained constantly low.

Testosterone administered to 22 patients induced a fall in serum PBI concentration in each case (Fig. 5). The PBI level averaged 4.7 μg. per 100 ml. before, and 3.0 μg. per 100 ml. during administration of testosterone (Table 3).

Studies carried out on 9 euthyroid males before and during testosterone

TABLE 2. SUMMARY OF DATA ON 5 SUBJECTS IN WHOM THE EFFECT OF TESTOSTERONE ON THYROXINE SURVIVAL WAS STUDIED

(Testosterone was administered as testosterone propionate, 50 mg. intramuscularly daily)

Subject		Thyroxine half-life (days)	
Name	Sex & Age	Before testosterone	With testosterone
R.H.	M 52	6.9	5.6
T.P.	M 66	5.2	5.4
A.K.	F 87	4.8	4.8
D.C.	M 75	7.8	7.8
J.R.	M 73	5.4	5.4

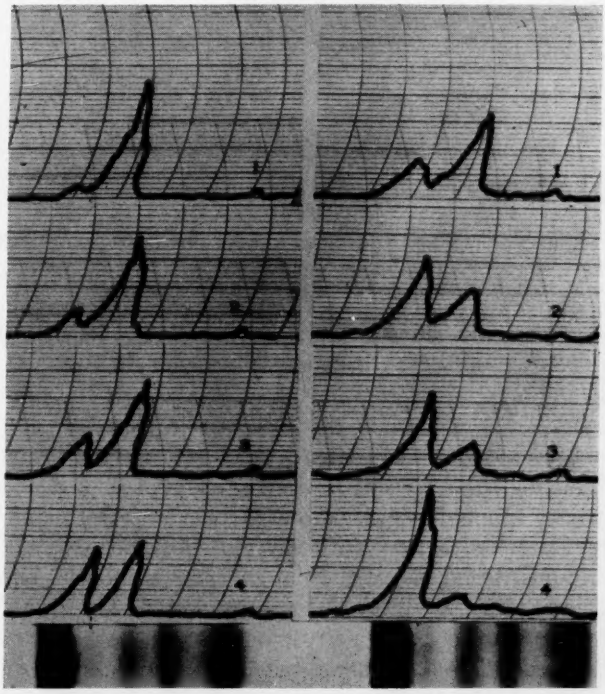

FIG. 4. Comparison of thyroxine binding by serum from an estrogen-treated subject (left) and a normal subject (right). The 4 scan-recordings indicate radioactivity on paper electrophoretic strips, the protein fractions of which are identified by the stained electrophoretograms below each series. The same quantity of radioactive thyroxine was added to each of the serum portions. Additional stable thyroxine brought the total amount of added thyroxine to 10 μg. per 100 ml. in portion 1, 48 μg. in portion 2, 85 μg. in portion 3 and 160 μg. in portion 4. The increased binding capacity of TBP is readily apparent in the recording on the left.

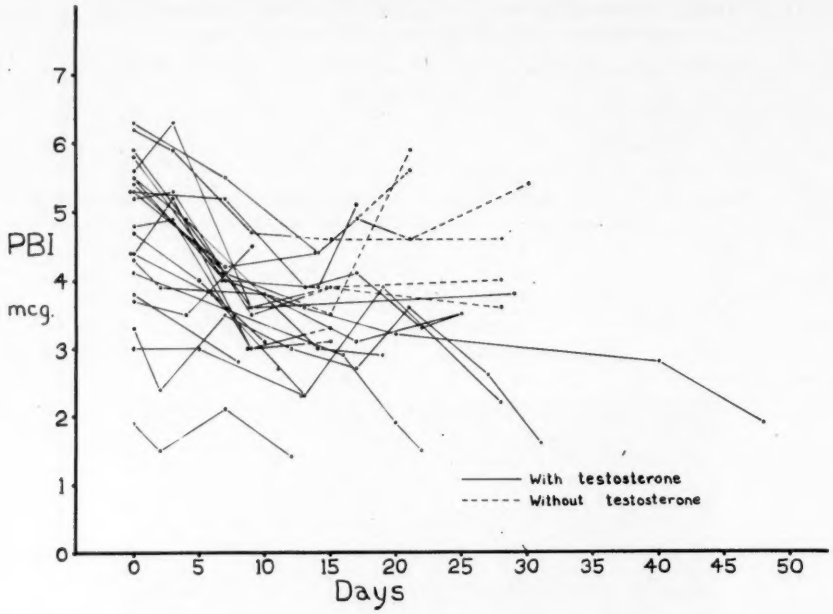

FIG. 5. Effect of administration of testosterone on the serum PBI level (μg./100 ml.) in 22 subjects.

therapy showed no change in the thyroxine-binding capacity of the serum in 6, and a decrease in 3. No consistent changes in the levels of serum albumin or globulin fractions were noted on analysis of the paper electrophoretic strips.

In 1 untreated hypothyroid patient, the administration of testosterone propionate, 50 mg. intramuscularly daily, resulted in a decrease in the binding capacity which, however, was still considerably greater than after adequate replacement therapy with thyroid substance. In this same patient, the administration of testosterone during replacement therapy with a daily dosage of 0.25 mg. of *l*-thyroxine resulted in a drop in the serum PBI level from 8.5 μg. to 4.6 μg. per 100 ml.; however, an alteration in thyroxine-binding capacity could not be demonstrated.

DISCUSSION

Although there is considerable information regarding the effects of estrogen and pregnancy on thyroid function, the sequence of events leading to the rise in serum PBI concentration has not been entirely clear. This may be due in part to differences between man and animals. Also, thyroidal

TABLE 3. SUMMARY OF DATA ON 22 SUBJECTS, SHOWING THE EFFECT OF TESTOSTERONE ADMINISTRATION ON THE SERUM PBI LEVEL

Subject		Methyl testosterone (mg./day, orally)	Testosterone propionate (mg./day, i.m.)	Serum PBI level (μg./100 ml.)	
Name	Sex & Age			Before testosterone	With testosterone (min.)
R.H.	M 52		50	5.3	4.2
M.M.	M 69		50	5.5	3.9
W.G.	M 73		50	5.4	3.5
L.Z.	M 24		50	6.3	4.4
E.G.	M 77		50	4.8	3.0
L.L.	M 59		50	4.4	3.0
I.L.	M 57		50	6.2	4.6
J.P.	M 80		50	5.6	3.6
O.S.	M 63		50	5.2	3.5
J.H.	F 41		50	3.8	2.6
E.N.	F 75	60		5.8	1.9
M.S.	F 82		30	4.3	3.1
J.T.	M 51		30	5.4	2.9
M.A.	F 93	60		3.0	2.2
E.H.	F 37		50	4.4	2.3
M.G.	F 88	50		5.3	3.3
B.H.	F 74	60		4.1	1.5
S.C.	F 75		50	4.7	2.7
E.A.	M 41		25	1.9	1.4
M.J.	M 50		25	3.3	1.6
P.B.	F 47	50		3.7	3.5
E.L.	F 62		50	5.9	3.6
				Avge. 4.7	*3.0*

economy in pregnancy may not be completely analogous to that in estrogen-treated subjects.

Our studies and those of Dowling *et al.* (4) indicate that the primary effect of estrogen is to increase the thyroxine-binding capacity of serum. This occurs at any level of thyroid function and is independent of the amount of available thyroxine. Whether there is a quantitative increase in TBP or an increase in the number of binding sites per protein molecule is unknown. Although characterization of TBP has been attempted, the fraction has not been isolated (11). The pronounced quantitative alterations in plasma proteins during pregnancy (12–14) suggest a quantitative rather than a qualitative change in TBP. We have observed no change in the PBI

level or binding capacity of serum from a normal male at one hour and at eighteen hours after the intravenous injection of 20 mg. of estrogen (Premarin), nor has the addition of Premarin to serum *in vitro* had any effect on binding capacity—also suggesting a quantitative rather than a qualitative change.

The increase in serum PBI concentration upon administration of estrogen to athyrotic subjects receiving replacement therapy indicates that a functioning thyroid gland is *not* essential for this phenomenon. No increase in the PBI level was induced in the untreated or inadequately treated hypothyroid patients in whom the thyroxine-binding capacity of the serum was *already* abnormally increased before administration of estrogen. In these patients with no, or a very limited, supply of thyroxine, the creation of more binding sites might not be expected to increase measurably the amount of bound thyroxine.

A consequence of the increased thyroxine-binding capacity is the increase in concentration of circulating thyroxine. When the supply of thyroid hormone is constant, as in adequately treated athyrotic patients, the rate of disposal of hormone must decrease. This is demonstrated by the increase in thyroxine survival time. The prolongation of thyroxine survival may indicate an increase in the quantity of circulating thyroxine (sequestration in the blood), or a decrease in the disposal of thyroxine, or both. If an increase in quantity of thyroxine were solely responsible, the change in the survival curve should be a gradual one paralleling the slow rise in serum PBI concentration over a period of weeks. The more prompt change in the slope of the curve in each case, and the exaggerated retention of thyroxine in 7 cases during the initial days of estrogen therapy, indicate a decreased rate of disposal in addition to an increased quantity of thyroxine in the serum.

The immediate cause of the decreased rate of disposal of thyroxine is not entirely clear. Although direct depression of tissue metabolism by estrogen (15) is a possible explanation, it is more likely that increased serum binding itself accounts for the decreased rate of disposal, because of altered blood-tissue gradient. Robbins and Nelson showed that, despite the increased serum PBI level in pregnancy, the amount of free thyroxine in the serum is decreased (16). There is evidence to suggest that the degree of thyroxine-binding is inversely proportional to the metabolic state of the subject; thus, in hypothyroidism thyroxine-binding is increased, whereas in hyperthyroidism it is decreased (10). Conversely, in hypothyroidism the amount of circulating free thyroxine is decreased and in hyperthyroidism it is increased. Crispell and co-workers arrived at similar conclusions about the availability of circulating thyroxine in various states of thyroid function, by studying the uptake of thyroxine by red blood cells from the serum

of euthyroid and hyperthyroid subjects (17–19). Similar observations, employing patients' serum and rat muscle, were made by Hamolsky, Ellison and Freedburg (20). The decrease in the rate of peripheral thyroxine degradation in hypothyroidism and the increase in hyperthyroidism (21, 22) may also reflect the degree of binding of thyroxine in the blood. The binding of thyroxine to cells apparently is an irreversible process (23).

Since administration of estrogen results in a decreased rate of disposal of thyroid hormone, the basal metabolic rate (BMR) might be expected to fall, at least initially. A previous study showed no appreciable change in BMR in 5 patients (24); however, Dowling *et al.* noticed symptoms of myxedema in 3 patients (4) and Sherwood noticed a decrease in metabolic rate in rats during administration of estrogen (25). Hyperthyroidism is frequently ameliorated with the onset of pregnancy, when estrogen levels rise and thyroxine-binding is increased (26).

In the normal subject given estrogen, or in the euthyroid pregnant woman, it is probable that even an undetectable fall in the metabolic rate stimulates an increased production of thyroid hormone, probably through the pituitary. Studies of thyroidal I^{131} uptake reflecting thyroidal activity in pregnant women indicate a consistently high I^{131} uptake throughout pregnancy, continuing for a short time into the postpartum period (27–29).

From our studies and those of others mentioned here, we arrive at the following sequence of events initiated by estrogen in a normal subject (intact pituitary and thyroid): 1) increased thyroxine-binding, 2) increased concentration of serum PBI, 3) decreased utilization of thyroxine, 4) decreased basal metabolic rate, 5) increased pituitary secretion of thyrotropin, and 6) increased thyroidal activity with a further increase in the serum PBI level. In considering these events, the essential role of the pituitary is questionable; however, in the intact organism it probably plays a significant part. Feldman's observations on rats indicate that administration of estrogen results in increased thyroidal activity despite the lack of a pituitary (30). He suggested a direct effect of estrogen upon the thyroid gland. A degree of thyroidal autonomy has been proposed by Dowling, Freinkel and Ingbar (4), who speculate that thyroidal activity may be influenced by the concentration gradient between thyroidal thyroxine and serum thyroxine. A decrease in circulating free thyroxine (or increased serum binding of thyroxine) should result in an increased output of hormone from the gland with a noticeable difference between primary and secondary hypothyroidism (22).

Our results regarding the effects of testosterone on the circulating thyroid hormone were less striking than those reported by others. A consistent fall in the PBI level was induced by testosterone administered either as the propionate or as methyltestosterone, in both males and females; however,

there was an inconsistent decrease in thyroxine-binding capacity. In 1 myxedematous patient, with TBP already increased, the decrease in binding capacity was considerable when testosterone was given. In the report by Keitel and Sherer, mention was made of a decrease in binding capacity upon administration of testosterone to 2 subjects (5). Federman, Robbins and Rall reported a striking decrease in binding capacity in the 4 subjects studied during administration of methyltestosterone (6).

With a fall in the serum PBI level, probably secondary to a decrease in thyroxine-binding by serum protein, more free thyroxine should be available with an increased rate of peripheral utilization and an increased metabolic rate. Several authors have reported that the basal metabolic rate increases during testosterone therapy (31–35). Likewise, an increased metabolic rate might be expected to result in an inhibition of pituitary thyrotropin secretion and a decrease in thyroidal activity. Keitel and Sherer reported a decreased thyroidal I^{131} uptake in 8 of 10 subjects given testosterone (5). As suggested by other investigators (5, 6), it appears that the effects of testosterone on thyroidal economy are the opposite of those occurring with estrogen, although the effects of testosterone are not so marked as those of estrogen.

These studies with estrogen and testosterone emphasize the importance of the mechanism for transport of thyroid hormone in thyroidal economy. Not only does this mechanism serve as a buffer between production and utilization of hormone, but it appears to play a dynamic role in regulating these functions. The role of this transport system in the pathologic physiology of thyroid disease has yet to be fully evaluated.

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COEXISTENCE OF ADDISON'S DISEASE AND DIABETES MELLITUS: REPORT OF THREE CASES WITH A DISCUSSION OF METABOLIC INTERRELATIONSHIPS

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ABSTRACT

Three cases of co-existing diabetes mellitus and Addison's disease are reported. In 2, diabetes developed after adrenal insufficiency had existed for years. Only 15 cases with this sequence of onset of the 2 diseases have been reported previously. The effect of each disease upon the pathophysiology and course of the other is discussed. The usual sequence of onset is diabetes mellitus complicated later by adrenal insufficiency. In such instances there occurs amelioration of the diabetic state, followed by progressive deterioration of the patient unless appropriate treatment is given for adrenal insufficiency. With such treatment the diabetes returns to its former degree of intensity, but stabilization is facilitated. When Addison's disease is complicated secondarily by diabetes mellitus, there may be intensification of the adrenal insufficiency which can be corrected only partially by larger doses of salt-active steroids. After appropriate therapy for the diabetes has been instituted, the steroid requirement returns to its former level. A brief survey is included of diseases other than those involving the adrenal cortex, which tend to ameliorate the diabetic state.

THE coexistence of Addison's disease and diabetes mellitus is rare. Since 1866 (1) only 67 cases have been reported. Webster and Hurt (2) tabulated 61 cases and added another one. Five more cases have been reported subsequently (3–6). Since both diseases exert profound and opposite effects on carbohydrate metabolism, their simultaneous existence offers a unique opportunity for study.

We have observed 3 cases of coexistent Addison's disease and diabetes mellitus. In 1 instance the adrenal insufficiency developed six years after the onset of the diabetes, in conformity with the usual sequence of onset. In the other 2 cases the Addison's disease preceded the development of the

Received January 2, 1959.

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diabetic state by fifteen and nine years respectively. (Addison's disease complicated secondarily by diabetes has been reported in only 15 cases.) It is the purpose of the present paper, 1) to record these 3 cases, especially since the 2 instances of Addison's disease followed by diabetes constitute a considerable percentage of the reported experience with this sequence of onset, and 2) to review the effects of each disease upon the abnormal physiology and course of the other.

CASE REPORTS

Case 1

L.W., a 38-year-old white male, was first admitted to the University Hospital in 1940 with the chief complaints of weakness and darkening of the skin. Three and a half years prior to admission the patient first noticed darkening of the skin of the exposed areas of his body. Several of the areas of increased pigmentation subsequently became depigmented. For eighteen months he had experienced progressive lassitude, asthenia and anorexia. Two months prior to admission, weakness had become so severe that he was admitted to another hospital, where treatment was given for adrenal insufficiency. Following discharge he was well until the evening before admission when he awoke from his sleep with convulsive movements associated with short periods of unconsciousness. He had lost 34 pounds since the onset of his illness. There was no history of diabetes mellitus or exposure to tuberculosis.

Physical examination revealed an emaciated white male, too weak to sit erect in bed. The blood pressure was 86/64 mm. Hg. There was diffuse tan pigmentation which was accentuated over the hands, feet, nipples and genitalia. There was no buccal pigmentation. Circular areas of vitiligo were present over the trunk and glans penis. No other physical abnormalities were noted. Laboratory study showed the urine to be normal. The hemoglobin level was 84 per cent and the white blood cell count 10,500 per cu. mm. with 46 per cent polymorphonuclear leukocytes, 46 per cent lymphocytes and 3 per cent eosinophils. The level of fasting blood sugar was 84 mg. per 100 ml., and of nonprotein nitrogen 36 mg. per 100 ml. Reaction to a tuberculin skin test was positive. X-ray examination of the chest showed minimal calcific fibrotic scarring of the left apex. Roentgenograms of the abdomen showed no calcification of the adrenal areas. A Kepler-Power-Wilder salt deprivation and potassium-loading test was performed. This precipitated an acute adrenal crisis. The patient responded to treatment and at the time of discharge was receiving 1 mg. of desoxycorticosterone acetate (DCA) intramuscularly and 10 Gm. of sodium chloride orally per day.

The patient was not seen again until 1947. He had been feeling well with the replacement therapy, but began gradually to discontinue it. His previous complaints of weakness and fatigue returned. The findings upon physical examination were similar to those at the time of the first admission, except that the areas of leukoderma were more pronounced. Levels of fasting blood sugar on two occasions were 29 mg. and 56 mg. per 100 ml. Replacement therapy was adjusted and the patient was discharged improved.

He was readmitted in 1949, two weeks after stopping injections of DCA and after development of cellulitis in the left thigh. Impending adrenal crisis was treated vigorously. The thigh abscess was incised and drained. On two occasions the level of fasting blood sugar was 250 mg. per 100 ml., and the urine contained 0.5 per cent glucose. An intravenous glucose tolerance test was performed using 50 cc. of 5 per cent glucose. The blood sugar values were: fasting, 266 mg.; half hour, 379 mg.; one hour, 338 mg.;

one and a half hours, 315 mg.; two hours, 274 mg.; two and a half hours, 257 mg.; three hours, 244 mg.; three and a half hours, 223 mg.; and four hours, 210 mg. per 100 ml. Upon discharge, the daily regimen was 1 mg. of DCA, 3 Gm. of sodium chloride, 12 units of protamine zinc insulin, and a 2800-calorie diet with 250 Gm. of carbohydrate. The fasting blood sugar level at this time was 95 mg. per 100 ml. An insulin tolerance test performed prior to discharge, using 0.1 unit of regular insulin per Kg. of body weight, yielded the following blood sugar values: fasting, 127 mg.; half hour, 75 mg.; one hour, 32 mg.; one and a half hours, 27 mg.; and two hours, 23 mg. per 100 ml. It was followed by a severe hypoglycemic reaction.

There were several subsequent readmissions to the University Hospital for impending adrenal crisis precipitated by upper respiration infections. The diabetes was difficult to control. Wide fluctuations of the blood sugar level occurred with daily doses of protamine zinc insulin varying between 6 and 10 units. In 1949, urinary excretion of 17-ketosteroids was 2.7 mg. per day. In 1950, cortisone therapy was started in dosages varying between 12.5 and 25.0 mg. per day. With this addition the diabetes became more severe and the daily insulin requirement rose to 26 units of NPH. However, better control of the diabetic state and greater stability were achieved. Prior to the institution of cortisone therapy a severe psychosis had developed. The patient was admitted to a mental hospital, where he later died. Autopsy (Dr. James French) revealed idiopathic atrophy of the adrenal glands and severe hypoplasia of the pancreatic islets. An active chronic fibrocaseous tubercle was found in the liver.

Case 2

F.N. was a 51-year-old white female, first seen at the University Hospital in August 1940 with a history of weakness, nausea and malaise of six months' duration. These symptoms had been gradual in onset, but for the prior three years the patient had noticed increasing pigmentation of the skin. She had lost 27 pounds in twelve months. She had also experienced attacks of marked weakness, diaphoresis and fainting that were relieved by ingestion of food. Amenorrhea had developed twelve months before admission. There was no family history of tuberculosis.

Physical examination revealed a darkly pigmented adult female who appeared both acutely and chronically ill. Vital signs were normal. Typical Addisonian pigmentation was present on the body and buccal mucosa. Pubic and axillary hair was sparse and the breasts atrophic. No other physical abnormalities were noted. Urinalysis showed a trace of albumin and 50 white blood cells per high-power field. The hemoglobin level was 16 Gm. per 100 ml. The white blood cell count was 8150 per cu. mm. with 46 per cent polymorphonuclear leukocytes, 51 per cent lymphocytes, 2 per cent basophils and 1 per cent monocytes. Reaction to a tuberculin test was positive. The chest was normal radiographically. The patient was treated with a high carbohydrate diet, added salt and buccal DCA. Marked improvement in symptomatology occurred, with a weight gain of 12 pounds. Attacks of weakness and trembling persisted in the morning whenever breakfast was delayed.

During the next seven years the patient was readmitted to the University Hospital several times for spontaneous and therapeutic abortions and minor surgical procedures. In 1948, because of increasing severity of the symptoms of hypoglycemia, the carbohydrate content of the diet was increased. The level of fasting blood sugar at that time was 60 mg. per 100 ml. (Somogyi-Nelson). In 1951 a right pyelonephritis developed, and 20 mg. of cortisone per day orally was started. This addition to the regimen resulted in complete alleviation of the hypoglycemic symptoms. During the next four years,

repeated episodes of urinary-tract infection occurred. Pyelographic and bacterial studies for renal tuberculosis yielded negative results.

In August 1955, the trimethyl ester of desoxycorticosterone (DOC) in an intramuscular dosage of 37.5 mg. per month was substituted for buccal DCA. Routine urinalysis showed 1 per cent glycosuria, but this finding had been overlooked. The patient returned in one month complaining of frequency of urination not associated with polydipsia. She had noticed a pronounced lack of pep and energy, postural vertigo, and a weight loss of 10 pounds. In view of the past history, the patient's complaint of frequency was ascribed to a recurrence of urinary-tract infection. It was believed that the other symptoms represented salt and water depletion from inadequate dosage of the DOC trimethyl ester. The dosage was increased to 50 mg. per month. Three weeks later the patient was admitted to the hospital complaining of nausea, vomiting, diarrhea, abdominal and leg cramps and generalized weakness. She stated that the last injection of DOC trimethyl ester caused improvement in her previous symptoms until one week prior to admission, when they had returned in intensified form. In addition, marked polydipsia had developed. There was no family history of diabetes mellitus. The results of physical examination were as previously noted, except for an increase in generalized pigmentation and dehydration. Urinalysis showed 1 per cent glucose. The blood glucose level was 325 mg. per 100 ml. and the following levels were found in the serum: sodium 131 mEq. per liter, chloride 96 mEq. per liter, potassium 4.5 mEq. per liter, CO_2 combining power 22 mEq. per liter, and nonprotein nitrogen 15 mg. per 100 ml. The patient was treated initially with intravenous saline, glucose and hydrocortisone. With recognition of the diabetes, she was given a 2000-calorie diet containing 200 grams of carbohydrate, and 15 units of NPH insulin daily. Four days following the beginning of therapy the level of fasting blood sugar was 156 mg. per 100 ml. One month following discharge the patient had gained 10 pounds and her previous symptoms had completely disappeared. Electrolyte balance was well maintained with 37.5 mg. of DOC trimethyl acetate once monthly, and cortisone was continued in a dosage of 30 mg. daily. Present therapy consists of 30 mg. of hydrocortisone, 0.1 mg. of 9-alpha-fluorohydrocortisone, and 36 units of Lente insulin each day. At one time the diabetes was controlled by tolbutamide (7); however, six months later, following another urinary-tract infection, the diabetes became and has remained resistant to this drug. After withdrawal of hydrocortisone for several days, urinary excretion of 17-hydroxycorticoids was 0.7 mg. per day.

Case 3

J.D. was a 65-year-old white male, first seen at the University Hospital in October 1953. Diabetes mellitus had been discovered in 1947, when a large carbuncle had developed on his neck, associated with severe polyuria, polydipsia and a 40-pound weight loss. The level of fasting blood sugar was 350 mg. per 100 ml. The patient had responded well to treatment and the diabetes had been controlled with diet alone. He had remained in apparent good health until the spring of 1953. He then noticed increasing asthenia, which was more intense in warm weather. Postural vertigo, anorexia and intermittent vomiting developed. There was a 30-pound weight loss. In June 1953, blue-black spotty pigmentation was noted on the mucous membranes of the lips. In September 1953, he was hospitalized in another institution, where x-ray examination of the chest demonstrated infiltration at the right lung base. Urinalysis showed a normal urine. Bronchoscopic biopsy findings were compatible with early tubercle formation. The lung lesion failed to clear and the patient was referred to the University Hospital for further study.

The past history disclosed that the patient had been aware of hypertension for many years. A systolic pressure of more than 200 mm. Hg had been recorded, and life insurance had been refused five years prior to hospitalization on this basis. There was no family history of diabetes or tuberculosis.

Physical examination revealed an adult male who appeared chronically ill. The blood pressure was 110/70 mm. Hg in the recumbent position with a reduction to 60/40 mm. on standing. Typical Addisonian pigmentation was present, involving the buccal mucosa, lips and hard palate. There was dullness to percussion over the right posterior lung base, and dry crackling rales. The remainder of the physical examination findings were normal. Urinalysis showed a trace of glucose. The hemoglobin level was 12.9 Gm. per 100 ml. and the white blood cell count was 7350 per cu. mm. with 38 per cent polymorphonuclear leukocytes, 31 per cent lymphcytes, 18 per cent monocytes, 10 per cent eosinophils and 2 per cent basophils. The serum nonprotein nitrogen level was 56 mg. per 100 ml., sodium 131 mEq. per liter, chloride 99 mEq. per liter, potassium 5 mEq. per liter, and CO_2 combining power 25 mEq. per liter. The level of fasting blood sugar on two occasions was 80 mg. and 98 mg. per 100 ml. (Somogyi-Nelson). X-ray examination of the chest revealed extensive pleural thickening on the right with pleural calcification. There was evidence of loculated empyema posteriorly. Abdominal x-ray examination showed linear calcification in the region of the left adrenal. The reaction to a tuberculin test was positive. No acid-fast bacilli were found in the sputum. An 8-hour intravenous infusion of 20 units of ACTH was given on two successive days. The baseline urinary 17-ketosteroid excretion was 2.4 mg. per day and the total eosinophil count, 511 per cu. mm. The 17-ketosteroid excretion during the two days of stimulation was 2.5 and 2.6 mg. per day; there was no significant reduction in the eosinophil counts. Treatment for adrenal insufficiency was instituted and the patient was discharged taking 25 mg. of cortisone orally and 4 mg. of DCA buccally per day. He received a diet of 1800 calories containing 150 grams of carbohydrate. At the time of subsequent visits the level of fasting blood sugar ranged between 72 mg. and 95 mg. per 100 ml. (Somogyi-Nelson).

The patient remained in good health until October 1955, when pain and tenderness developed in the right lateral chest. Subsequently a nontender swelling of the chest wall measuring 4 inches in diameter appeared. There was a return of asthenia and anorexia with a weight loss of 10 pounds in six months. A diagnosis of empyema necessitatis, probably tuberculous in origin, was made and hospitalization advised. Chest roentgenograms revealed a marked increase in the previously noted abnormality at the right lung base, consistent with a loculated empyema. Acid-fast bacilli were present in the sputum. Antituberculosis medication was started. Two months following admission to the hospital and after a standard preparatory diet, a glucose tolerance test showed the following blood sugar values: fasting, 83 mg.; half hour, 123 mg.; one hour, 160 mg.; one and a half hours, 198 mg.; two hours, 183 mg.; three hours, 165 mg.; and four hours, 97 mg. per 100 ml. Because of failure to improve with conservative management, thoracotomy with drainage of the empyema cavity and partial decortication was performed by Dr. Cameron Haight. The patient was prepared for surgery with intramuscular hydrocortisone. He withstood the operation well and has been maintained with oral hydrocortisone and 9-alpha-fluorohydrocortisone. The daily dosage of hydrocortisone was 25 mg. up to December 1957, and 30 mg. since that time. In December 1957 the level of fasting blood sugar was 144 mg. per 100 ml., and in April 1958 it was 214 mg. per 100 ml. in spite of an 8-pound weight loss. Addition of tolbutamide to the diabetic dietary regimen resulted in a normal fasting blood sugar level.

DISCUSSION

The total number of reported cases of co-existing Addison's disease and diabetes mellitus has now reached 70. Up to the year 1949, a review of the literature (8) revealed only 24 such cases. The increasing number reported during the past ten years is undoubtedly a reflection of more frequent clinical recognition of Addison's disease.

In most instances, the diabetes has preceded the adrenal insufficiency. In such cases the ameliorating effect of adrenocortical insufficiency upon diabetes mellitus has been striking. The situation is analogous to that demonstrated first by Long and Lukens (9) in the depancreatized adrenalectomized cat.

Adrenocortical insufficiency should be suspected whenever a diabetic patient manifests a sharp decrease in total insulin requirement together with great instability of the blood sugar level and severe hypoglycemic episodes. Resistance to ketosis and hypoglycemia unresponsiveness are confirmatory findings. Despite amelioration of the diabetic state, the patient shows the progressive deterioration characteristic of untreated Addison's disease. Administration of physiologic amounts of carbohydrate-active steroids restores the previous intensity of the diabetic state, resulting in a sharp rise in the concentration of blood sugar, increased urinary loss of sugar, increased insulin requirements, diminished insulin sensitivity, increased nitrogen excretion, and a return of susceptibility to ketosis (10). Although replacement therapy for adrenal insufficiency in the patient with co-existing Addison's disease and diabetes mellitus produces intensification of the diabetic state, it imparts greater stability and results in easier management of the diabetes.

The effects of adrenocortical insufficiency upon carbohydrate metabolism appear to be directly related to the associated hydrocortisonopenia. Of all the known steroidal hormones of the adrenal cortex, only hydrocortisone, when given in physiologic quantities, is capable of influencing carbohydrate metabolism significantly (11). It increases gluconeogenesis from protein precursors and inhibits peripheral utilization of glucose (11–14). About 30 per cent of patients with primary aldosteronism exhibit decreased tolerance for carbohydrate (15) despite normal production of hydrocortisone. However, neither aldosterone nor corticosterone (16) in physiologic amounts exert important effects upon carbohydrate metabolism.

The development of diabetes mellitus in a patient with pre-existing Addison's disease is a much less common sequence of events. In contrast to the improvement in carbohydrate metabolism that occurs when adrenal insufficiency develops in a diabetic, the development of diabetes in a patient

with previously well-controlled Addison's disease is characterized by an apparent exacerbation of the adrenal insufficiency. In such cases there is a return of anorexia, lassitude, loss of weight, and low blood pressure. One is tempted simply to increase the amount of steroid replacement therapy, unless one realizes that it is the osmotic diuresis provoked by intense glycosuria which causes secondary renal losses of salt and water. Increasing the dosage of carbohydrate-active steroid does not correct the situation and may actually magnify it by augmenting the glycosuria. Large doses of salt-retaining steroids are likewise relatively ineffective in preventing further losses of salt and water. However, when the diabetes is recognized and appropriate treatment with insulin and diet is instituted, control of the Addison's disease is readily achieved with replacement therapy similar to that required prior to the onset of the diabetes.

It has been suggested that an unexplained amelioration of the diabetic state should cause suspicion of co-existing adrenal insufficiency. This situation is observed, however, in other instances of both endocrine and non-endocrine organ failure and serves to emphasize the multiple extrapancreatic factors which may effect the blood sugar level. Thus, hypothyroidism has been reported to cause improvement in diabetes mellitus (17, 18). The amelioration of diabetes observed in hypopituitarism (19) can be attributed in part to secondary adrenocortical and thyroidal failure, but decreased secretion of growth hormone is presumably an additional factor. Hepatic failure (20) has a variable effect on carbohydrate metabolism; improvement of the diabetic state has been reported subsequent to the development of cirrhosis of the liver, whereas extreme lability of the diabetes is often found in hemochromatosis. The development of intercapillary glomerulosclerosis has also been noted to cause a decrease in insulin requirements both clinically (21) and experimentally (22), but this phenomenon requires further clarification (23). One of us has recently observed amelioration of diabetes mellitus during the development of an insulinogenic tumor of the pancreatic islets (24).

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CHANGES IN DIABETES INSIPIDUS DURING PREGNANCY AND LACTATION

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ABSTRACT

A patient with diabetes insipidus repeatedly demonstrated an increase in the requirement for vasopressin during pregnancy, a decrease during lactation, and a less striking increase during the latter part of the menstrual cycle. Results of water deprivation tests on this patient suggested that endogenous antidiuretic hormone was released during lactation. Intravenous infusions of small doses of vasopressin had a pronounced antidiuretic effect on the third and twenty-eighth days of the menstrual cycle, a distinctly smaller effect during pregnancy, and a possibly smaller effect on the eighteenth day of the menstrual cycle.

DESPITE the striking diuresis observed in patients with diabetes insipidus it is known that the defect in antidiuretic hormone production in many of these patients is incomplete, since antidiuretic responses can be elicited with certain stimuli such as the administration of nicotine (1, 2). Moreover, variations in diuresis are observed with changes in the function of other endocrine glands, particularly the adrenal and thyroid (3–5). Little is known concerning the interrelationship between diabetes insipidus and the endocrine changes in pregnancy, lactation, and the menstrual cycle (6).

The present report concerns a patient with diabetes insipidus in whom the disorder underwent striking changes during pregnancy and lactation, and at various phases of the menstrual cycle.

CASE REPORT

The patient, a 33-year-old white female, gravida 10, para 8, has been under observation since 1951. She first noted polydipsia and polyuria coincident with menarche at age 13. Without treatment she drank between 10 and 15 quarts of fluid each day. At age 20, a diagnosis of diabetes insipidus was made. Administration of posterior pituitary powder by nasal insufflation produced relief of symptoms. However, this was discontinued after three years because of nasal irritation, and symptoms returned. When she

Received January 29, 1959.

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was first seen in 1951 at age 26, the patient complained only of polydipsia and polyuria. The past history included a hospitalization for pulmonary tuberculosis between the ages of 12 and 15 years and excision of a tuberculous leg ulcer at age 28. At age 15, after the onset of diabetes insipidus, she suffered a head injury and was unconscious for three days. The patient's father, mother, and one sister died of tuberculosis. There was no history of diabetes insipidus in other members of the family.

In 1951 there had been no abnormal physical findings. Skull roentgenograms, an electroencephalogram and results of spinal fluid examinations were normal. A roentgenogram of the chest showed fibro-calcific densities in the right upper lobe, which were interpreted as healed tuberculosis. An intravenous pyelogram indicated moderate enlargement of the bladder, ureters and renal pelves. A Carter-Robbins test (7) was performed. After the oral administration of 1100 ml. of water, urine flow increased to 21 ml. per minute. The intravenous administration of 630 ml. of 2.5 per cent sodium chloride increased the flow further to 34 ml. per minute. Subsequently 2 units of vasopressin intravenously caused a decrease in the flow of urine to 4 ml. per minute.

In 1951 the patient had been instructed to take intramuscular Pitressin Tannate in Oil® (P.T.O.) and had done so for the seven years prior to the present entry. The frequency with which she took this medication was determined by the length of time that a dose of 5 units controlled her symptoms. When not pregnant she ordinarily required P.T.O. every four to five days. Menstruation was regular and occurred every twenty-eight days. She noted that late in the cycle she required vasopressin more often, and that an injection taken during menstruation might be effective for six to eight days. Since the beginning of P.T.O. therapy in 1951, she had been observed during 6 pregnancies. One pregnancy terminated in spontaneous abortion. During 3 pregnancies (1952, 1956 and 1957) she noted an increase in the requirement for P.T.O. to 5 units daily. In 1953 she noticed a less marked increase in the requirement for P.T.O. while she was pregnant, and in 1954 she noted no change during pregnancy. She had lactated briefly after each pregnancy and required no P.T.O. or other forms of vasopressin while she was lactating. The patient reported that a further decrease in urine volume occurred shortly after nursing. In 1956 she nursed her child for ten days and did not require vasopressin for twenty days post partum.

The patient was admitted for study to the Syracuse University Hospital in August 1957, when she was 7½ months pregnant. On physical examination she appeared to be a normal woman in her seventh month of pregnancy. At this time the diuresis could not be controlled with 5 units of P.T.O. daily. Despite administration of 5 units of aqueous vasopressin subcutaneously every six hours, the flow of urine averaged 10 ml. per minute (14 liters per day).

Laboratory findings at this time included a hemoglobin level of 12.2 Gm. per 100 ml., hematocrit 37 per cent, and white blood cell count 8800 per cu. mm. The urine had a specific gravity of 1.002, and contained no albumin, sugar or acetone. There were 10 to 20 white blood cells per high-power field in the centrifuged urinary sediment. The excretion of phenolsulphonphthalein was 10 per cent in fifteen minutes and 80 per cent in two hours. The level of fasting blood glucose was 74 mg. per 100 ml., and of nonprotein nitrogen 18 mg. per 100 ml. The blood Wassermann reaction was negative.

Special studies: In an attempt to explain the variations in the state of diabetes insipidus in this patient, studies were performed of the response to deprivation of water and to infusion of vasopressin during pregnancy, during lactation, and subsequently at various intervals in the menstrual cycle. In the water deprivation test (8) the response to a four-hour period of thirsting was studied at a time when the patient had not taken

vasopressin for at least four days. The osmolality of the urine was determined at hourly intervals, and of the serum at the beginning and at the end of the period. These measurements, expressed in milliosmols per Kg. of serum water or urine water, were obtained by the freezing-point depression method using a Fiske osmometer. In a subject without diabetes insipidus, deprivation of water leads to a rapid increase in the osmolality of the urine above that of the serum, while the initial serum osmolality (normally 285 ± 10 mOsm./Kg.) undergoes little change. In patients with severe diabetes insipidus, urine osmolality will not exceed serum osmolality during dehydration, and the serum may become considerably more concentrated because of the inability of the kidneys to conserve water.

The measurement of sensitivity to vasopressin (9) was carried out on four occasions, two of which were on the same day as the water deprivation test. The patient was first hydrated and the rate of spontaneous water diuresis was observed. Vasopressin[1] was then administered by a constant infusion pump at rates of 1 to 24 milliunits (mu) per hour. The rate of water diuresis before and during administration of vasopressin is expressed in the terms of the free water clearance (C_{H_2O}) which is calculated as the difference between urine flow (V) and osmolal clearance (C_{osm}) as follows:

$$C_{H_2O} = V - C_{osm}$$

where

$$C_{osm} = \frac{(U_{osm})\ V}{P_{osm}}$$

and represents the volume of fluid that would contain the urinary solutes in a solution isosmotic with the plasma. A positive value for C_{H_2O} indicates the degree to which water is being excreted in excess of solute, and a negative value indicates the degree to which water is being reabsorbed in excess of solute.

RESULTS

The water deprivation tests (Table 1) performed during pregnancy and on the eighteenth and twenty-eighth days of the menstrual cycle showed inability to concentrate the urine despite considerable loss of water and an

TABLE 1. WATER DEPRIVATION TESTS

Date	Condition of patient	Urine osmolality (mOsm./Kg.)		Urine flow (ml./min.)		Serum osmolality (mOsm./Kg.)	
		Initial	4th hour	Initial	4th hour	Initial	4th hour
Sept. 17, 1957	Pregnant, 8th month	65	100	13.9	11.1	289	299
Oct. 6, 1957	Lactating, 3 days post partum	177	463	7.7	1.4	283	—
May 16, 1958	18th day of menstrual cycle	95	144	14.1	8.7	287	300
July 12, 1958	28th day; menses began within 24 hrs.	75	83	8.5	7.2	273	277
Approximate values for normal subjects undergoing water diuresis just prior to water deprivation.		60–150	>300	>8	<2	Little change	

[1] A single lot of Pitressin® (Lot S216F), a mixture of hog and beef posterior pituitary extracts, was used; it had been kept refrigerated since it was reassayed on August 11, 1955. Potency at this time was 24 pressor units per ml. The assays were kindly performed by Mr. L. G. Rowe at the request of Dr. D. A. McGinty of Parke, Davis & Company.

TABLE 2. VASOPRESSIN SENSITIVITY TESTS

Date	Condition of patient	No vasopressin		Vasopressin (4 mu*/hr.)		Vasopressin (8 mu*/hr.)	
		C_{H_2O} (ml./min.)	C_{osm}	C_{H_2O} (ml./min.)	C_{osm}	C_{H_2O} (ml./min.)	C_{osm}
Aug. 30, 1957	Pregnant, 7th month	+15.9	3.6	—	—	+6.6	4.6
Mar. 26, 1958	3rd day of menstrual cycle	+10.7	4.9	−0.6	2.1	−0.6	1.4
May 10, 1958	18th day of menstrual cycle	+ 9.1	4.8	+4.9	2.7	+0.9	1.9
July 12, 1958	28th day of menstrual cycle	+ 7.3	2.8	−0.1	1.8	—	—

* Milliunits.

increase in serum osmolality. In contrast, water deprivation during lactation induced an increase in urine osmolality to 463 mOsm. per Kg. and a decrease of urine flow to 1.4 ml. per minute. Unfortunately the final serum osmolality determination was not obtained.

Studies of the response to exogenous vasopressin (Table 2) showed that the patient was very sensitive on the third and twenty-eighth days (less than twenty-four hours before onset of the menses) of the menstrual cycle, in that a dose of 4 ml. per hour induced conservation of water (a negative value of C_{H_2O}). At these times the patient usually required injections of P.T.O. at intervals of four to five days. On the eighteenth day of the menstrual cycle, a time when she frequently experienced an early escape from the action of P.T.O., there was apparently a decrease in vasopressin sensitivity, as C_{H_2O} remained positive at doses of 4 and 8 milliunits per hour. During pregnancy there was considerable decrease in the sensitivity to vasopressin; C_{H_2O} remained positive (6.6 ml. per minute) during the administration of 8 milliunits of vasopressin per hour. The administration of 24 milliunits per hour at this time decreased C_{H_2O} to +0.1 ml. per minute.

DISCUSSION

This patient with diabetes insipidus demonstrated an increase in the requirement for vasopressin during repeated pregnancies and a decrease during lactation. In addition there is suggestive evidence of an increased requirement for vasopressin during the second half of the menstrual cycle. The clinical observations were confirmed by changes in the response to deprivation of water and the intravenous infusion of vasopressin. The effect of coexisting pregnancy on diabetes insipidus has been the subject of many reports, with varying findings (10–13). Enhancement of the severity of diabetes insipidus might be expected for several reasons. Increased secretion of adrenocortical hormones during pregnancy (14) might antagonize antidiuretic hormone by increasing its inactivation, decreasing its release, or by an undefined action on the renal tubules (15). Increased activity of

adrenocortical and thyroid hormones during pregnancy might cause an increase in the excretion of urinary solutes. Augmented solute excretion would not only increase the flow of urine, but might also increase the requirement for vasopressin (4, 16). Increased plasma inactivation of oxytocin during pregnancy has been reported (17, 18); an analogous mechanism might inactivate antidiuretic hormone. Another possible explanation for the enhanced severity of diabetes insipidus during pregnancy is suggested by our observation of an increased requirement for vasopressin not only during pregnancy but to a lesser extent during the second half of the menstrual cycle; an increase in circulating progesterone is known to occur at these times. The occasional observation of improvement in diabetes insipidus during pregnancy is best explained by a decrease in the excretion of urinary solutes due to dietary restriction or vomiting.

Alexander and Downs (12) previously noted clinical improvement in diabetes insipidus during lactation. Our patient improved to the extent that she was able to maintain a small urinary volume for twenty days without the administration of vasopressin and was able to concentrate her urine in response to deprivation of water. These data suggest that endogenous antidiuretic hormone production reappeared during lactation, but they do not rule out the additional possibility that sensitivity to antidiuretic hormone might also have changed. In diabetes insipidus the hypothalamic-neurohypophyseal system may not be completely destroyed, and under certain conditions antidiuretic hormone secretion may reappear (19). There has been controversy regarding the separate secretion of antidiuretic and oxytocic activity from the neurohypophysis. There are reports (20–22) that antidiuretic and oxytocic substances can be secreted in markedly different proportions, though recent studies in the rat by Heller (23) have not confirmed this finding. During suckling, when there is marked liberation of the oxytocic hormone, there is also antidiuresis (24–26). Although this might represent the antidiuretic activity which is found in small amounts even in synthetic oxytocin, it is more likely due to the simultaneous release of antidiuretic hormone. In an animal experiment which is analogous to the present case, Benson and Cowie (27) found that ablation of the posterior lobe of the pituitary in a lactating rat abolished ejection of milk and caused diabetes insipidus. Subsequently, when these rats became pregnant again, the ability to eject milk was restored but diabetes insipidus persisted. In these animals there was no significant amelioration of diabetes insipidus during lactation. This may have been due to the more complete removal of sources of antidiuretic hormone in the experimental animals than in our patient, further suggesting that the source of antidiuretic activity during lactation is not oxytocic hormone itself.

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JCEM

IDENTIFICATION OF SUBSTANCE S IN HUMAN ADRENAL VENOUS BLOOD

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ABSTRACT

Use of the methylcyclohexane:dimethylformamide paper chromatographic solvent system resulted in the separation of 17-hydroxydesoxycorticosterone from corticosterone isolated from human adrenal venous blood. An unknown substance was also indicated. The amounts of 17-hydroxydesoxycorticosterone averaged 0.77 μg. per ml. of blood, whereas corticosterone was present in amounts averaging 2.8 μg. per ml. A comparison of the amounts of these substances with the amounts of hydrocortisone was also made.

ALTHOUGH results of recent studies (1–3) suggest that human adrenal venous blood contains at least 12 detectable steroids, only 3 of these have been identified, namely, hydrocortisone, corticosterone and 11-β-hydroxyandrostenedione (1). The presence of pregnene-3α,17α,21-triol-20-one (tetrahydro S) in urine (4, 5) led to speculation regarding the origin of this substance, which presumably might derive from reduction of Δ^4-pregnene-17α,21-diol-3,20-dione (substance S) in the liver. Forchielli and co-workers (6, 7) showed that tetrahydro S and 11 other metabolites resulted from the metabolism of substance S by rat liver homogenates. The purpose of this paper is to describe a new paper chromatographic solvent system — methylcyclohexane:dimethylformamide — for separating substance S from corticosterone, and the identification of substance S in adrenal venous blood from 9 hypertensive and 1 normotensive patient. A comparison with steroids from human adrenal tissue incubates is also made.

PROCEDURES

Materials

The blood samples were drawn from the adrenal vein prior to adrenalectomy. Adrenalectomy was performed for the treatment of hypertension. The clinical aspects of the patients have been discussed in detail in a previous communication from this laboratory

Received February 9, 1959.

(8). Patients undergoing removal of the second adrenal (stage II) were given 50 mg. of cortisone the night prior to operation and 50 mg. on the morning of the operation.

Methods

The blood immediately after withdrawal was hemolyzed by addition of an equal volume of water. Precipitation of the proteins by addition of 4 volumes of acetone was followed by filtration and evaporation of the filtrate in a vacuum below 45°C. The aqueous residue was extracted with ethyl acetate twice and then twice with chloroform. The residue after removal of solvents was subjected to paper chromatography by the basic methods that were described previously (9). The systems toluene:propylene-glycol (T:PG), methylcyclohexane:propylene-glycol (MC:PG) and the new system methylcyclohexane:dimethylformamide (MC:DMF) were used. In the new system the usual Zaffaroni technique was employed. The strips were saturated with dimethylformamide (stationary phase), the substance or extract applied to the starting line, and the strip resaturated with stationary phase only below the starting line. Descending development with methylcyclohexane saturated with dimethylformamide was carried out at room temperature for the predetermined length of time.

Fraction X represented material remaining on the paper chromatogram after development in T:PG until a 15-ml. volume of effluent per half-inch width of paper was collected. This effluent was evaporated and rechromatographed in the same system until a 3-ml. volume of effluent per half-inch width was collected. The chromatogram (fraction Y) obtained showed one double spot containing corticosterone (B) and 17-hydroxydesoxycorticosterone (S). Elution of this spot and rechromatography in MC:DMF for seventy-two hours resulted in good separation of S and B. In this system, B has a mobility of 0.59 compared to S taken as unity. Cortisone has a mobility of 0.16 under the same circumstances. Further chromatography of the effluent of fraction Y in MC:PG afforded separation of some of the least polar metabolites. 11-β-Hydroxyandrostenedione remained on the paper after collection of 17 ml. of effluent per half-inch width of paper.

Identification procedures

The mobility in several different solvent systems was determined. The acetate was prepared and its mobility checked. The indicated chemical reactions such as blue-tetrazolium reduction, the Porter-Silber test for 17-hydroxylation, and bismuthate oxidation were carried out. The spectrum in sulfuric acid was determined in all cases. With hydrocortisone (F) and corticosterone (B) sufficient material was available for determination of the melting point.

RESULTS

This report deals mainly with identification of the material in the zone of the second chromatogram (fraction Y) known to contain B and other substances. Figure 1 shows the results of scanning the chromatograms of the first 3 fractions in ultraviolet light in a Beckman spectrophotometer at 245 mμ by the technique described by Tennent *et al.* (10). Results regarding identification of other material in these chromatograms will be reported later. The identification of 11-β-hydroxyandrostenedione from human adrenal incubates has been previously reported (11). The major zone which absorbed ultraviolet light at 245 mμ in the second (Y) chromatogram was eluted and rechromatographed in the MC:DMF system. Figure 1 shows

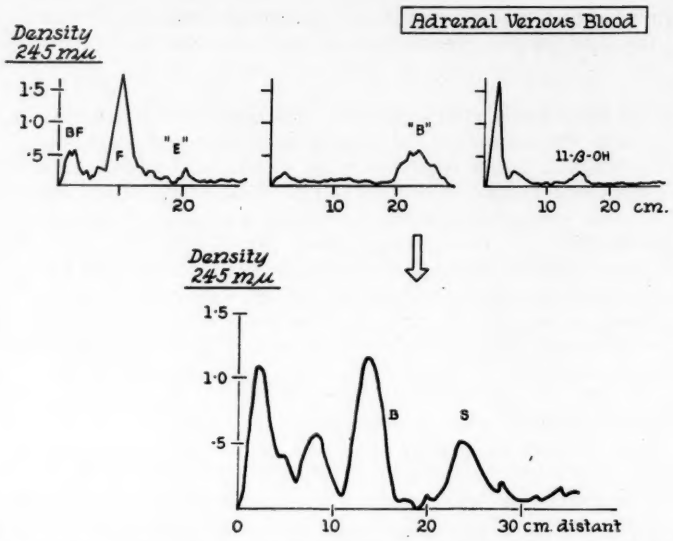

FIG. 1. Chromatographic pattern of extracts of adrenal venous blood. (Lower curve represents the methylcyclohexane:dimethylformamide chromatogram.)

the result of this chromatography of the eluted zone. There appeared 3 substances absorbing ultraviolet light at 245 mμ. Properties of these substances were as follows:

Properties of the substance "before B"

The most polar of the 3 zones seen in the MC:DMF chromatogram of the eluted B area (Fig. 1) absorbed ultraviolet light maximally at 240 mμ, as did the other 2 substances. No color was produced with alkaline blue-tetrazolium reagent, indicating a lack of the 20-keto-21-hydroxy substituent. The mobility of the substance was the same as that of 21-desoxyhydrocortisone in 3 different solvent systems. An acetate was formed on treatment with acetic-acid anhydride in pyridine at room temperature, which 21-desoxyhydrocortisone did not show. The absorption spectrum of the substance in sulfuric acid at 220–600 mμ yielded a curve (Fig. 2) which differed from that produced by 21-desoxyhydrocortisone under equivalent conditions. Oxidation with sodium bismuthate in the cold did not produce a 17-ketosteroid. Lack of material has prevented further characterization of this substance.

Identification of corticosterone

After chromatography in MC:DMF, the middle zone of the chromatogram absorbed ultraviolet light at 240 mμ to a degree indicating a quan-

tity which agreed with the amount calculated from the density of the blue color produced by reaction with blue tetrazolium. The acetate produced by reaction with pyridine and acetic anhydride at room temperature had the same mobility as authentic corticosterone acetate in the MC:PG system. The absorption spectrum of the free alcohol and its acetate in sulfuric acid at 200–600 mμ agreed with the spectrum produced by corticosterone under equivalent conditions. The fluorescence spectrum in sulfuric acid

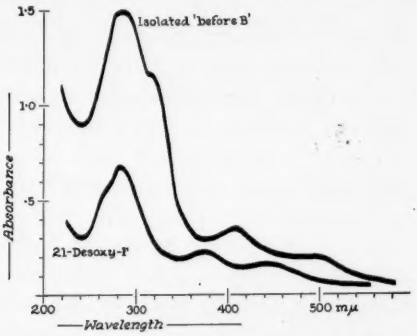

FIG. 2. Absorption spectrum in sulfuric acid of unknown substance from adrenal venous blood.

was the same as that of authentic corticosterone. The corticosterone from several adrenal blood samples was combined, acetylated and purified by chromatography on silica gel and recrystallized from acetone-methylcyclohexane. The melting point, 146–150°C., was not depressed on admixture with authentic corticosterone acetate.

Corticosterone has been isolated in crystalline form from adrenal venous blood by Romanoff *et al.* (1). Its presence in blood has also been indicated by Bush (2) and Hudson and Lombardo (3) as well as by Sweat (12).

Identification of 17-hydroxydesoxycorticosterone (substance S)

The least polar of the 3 zones in the MC:DMF chromatogram showed absorption at 240 mμ to a degree that coincided with quantitative values obtained by colorimetry of the blue-tetrazolium reduction product and the Porter-Silber phenylhydrazine reaction. The acetate formed by reaction with pyridine and acetic anhydride at room temperature had a mobility in MC:PG equivalent to that shown by authentic S acetate. The scarlet color shown by S in sulfuric acid was present. The absorption spectrum of the acetate in sulfuric acid at 220–600 mμ was identical with the spectrum of authentic S acetate (Fig. 3). Oxidation with sodium bismuthate at room temperature provided a substance (17-ketosteroid) producing a blue color with the Zimmermann reagent and possessing a mobility in MC:PG the same as that of Δ^4-androstene-3,17-dione. The absorption spectrum in sul-

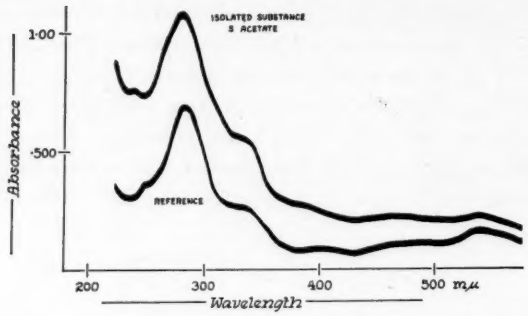

FIG. 3. Absorption spectrum in sulfuric acid of acetylated substance S isolated from adrenal venous blood.

furic acid of this oxidation product was identical with that shown by Δ^4-androstenedione under equivalent conditions.

The mobilities of substance S and compound B in the systems T:PG and MC:PG were similar and good separation was not possible. The mobility of B acetate as compared to S acetate in the MC:PG system was such that separation was not achieved. Due to lack of material, infrared spectrometry was not performed.

In Table 1 are listed the results of quantitative determinations of the amounts of corticosterone and substance S present in human adrenal venous blood. These measurements were made from extinction coefficients of the absorption of ultraviolet light at 240 mμ. Substance S was present in measurable quantities in all the bloods studied, and the concentration

TABLE 1. STEROIDS IN HUMAN ADRENAL VENOUS BLOOD

Subject	Stage of adrenalectomy	Steroid (μg./ml.)		
		Compound *F*	Compound *B*	Substance *S*
Ba	2*	17.0	3.8	2.0
Wz	2	11.7	2.0	0.6
Mc	1	10.5	6.2	—
Gre	2	12.4	1.6	0.4
Gr	2	8.3	0.9	0.2
Pros #1	2	12.4	3.0	1.0
Pros #2	2	6.4	3.0	0.8
Dr	2	5.7	2.8	0.7
We	1	6.5	0.3	0.4
Eng	2	11.0	5.0	1.6

* Patient undergoing removal of second adrenal.

averaged 0.77 μg. per ml. of blood. The amounts of hydrocortisone (average level, 10.2 μg. per ml.) in the same blood samples are listed for comparison.

Preliminary determinations of the amounts of substance S and compound B in incubates of adrenal tissue indicate that the proportion of S is lower than in the venous blood extracts. The substance more polar than B was also present in smaller amounts. This may indicate a more complete conversion of substance S to hydrocortisone by the incubates.

DISCUSSION

Substance S has not previously been shown to be a constituent of human adrenal venous blood. The paper chromatography system methylcyclohexane:dimethylformamide facilitated the separation of compound B from substance S as well as from one other unidentified metabolite. Eberlein and Bongiovanni (13) recently reported the finding of substance S in the peripheral blood of an 8-year-old male pseudohermaphrodite with congenital adrenal hyperplasia and systemic hypertension. No 11-hydroxylated steroids were found in this subject. Substance S has also been found in the adrenal venous blood of dogs by Farrell and Lamas (14). From these studies it appears that substance S is produced by the adrenal. In adrenal carcinoma it apparently is produced in excessive amounts which are eliminated in the urine as tetrahydro S, as has been reported by us (4) and by Rosselet *et al.* (5).

The blood samples used in the present study were drawn at the time of adrenalectomy. This may involve a hyperactive adrenal at the time of the withdrawal of blood, and thus may not present the normal picture of adrenal steroid production by the subject. The patients in these studies received 50 mg. of cortisone daily. At this time one can only speculate on the possibility that the cortisone may have been the precursor of the isolated substance S or may have caused an inhibition of the 11-hydroxylating enzyme system in the adrenal tissue.

Acknowledgments

This work was supported in part by U. S. Public Health Grant CY-3644, a grant from the Southeastern Pennsylvania Heart Association, and Institutional Grant 64-D of the American Cancer Society.

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URINARY EXCRETION OF β-CORTOLONE (3α,17α, 20β,21-TETRAHYDROXYPREGNANE-11-ONE) IN YOUNG AND ELDERLY MEN AND WOMEN*

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ABSTRACT

β-Cortolone (3α,17α,20β,21-tetrahydroxypregnane-11-one) was found as a regularly occurring metabolite in the urine of 20 young and elderly subjects of both sexes. The range of excretion values for young subjects was 0.3 to 2.9 mg. per twenty-four hours, and that for elderly subjects was 0.4 to 1.0 mg. per twenty-four hours. When expressed as a function of creatinine excretion, β-cortolone excretion was not significantly altered by age. It was, however, significantly correlated ($r = 0.91$) and proportional (20.1 per cent ±1.5) to the urinary excretion of tetrahydrocortisol, allotetrahydrocortisol and tetrahydrocortisone.

FUKUSHIMA and associates have (1) described the isolation of cortolone (3α,17α,20α,21-tetrahydroxypregnane-11-one) and β-cortolone (3α,17α,20β,21-tetrahydroxypregnane-11-one) as well as the respective "cortol" epimers from human urine after administration of adrenocorticotropin (ACTH). They also found cortolone in the urine after administration of 100 mg. of cortisone to a human subject. We have previously observed the regular occurrence of urinary metabolites which react positively to a test for glycerols on paper chromatograms and which are more polar than tetrahydrocortisol, in extracts of urine from normal subjects (2). Recently we attempted to measure quantitatively the cortolone component in extracts of urine from 5 young males, 5 young females, 5 elderly males and 5 elderly females, and to compare the excretion of cortolone with that of tetrahydrocortisol (THF), allotetrahydrocortisol (ATHF) and tetrahydrocortisone (THE) (3–5).

METHODS

The 10 young subjects were laboratory personnel. The 10 elderly subjects were residents of homes for the aged, and were in reasonably good health; no medications were given during collection periods. Methods for incubation and extraction of urine and paper chromatography were similar to those previously described for THF, ATHF and THE metabolites (3, 4) with the exception that the 12-hour urine aliquots of the

Received February 19, 1959.

* Aided by U. S. Public Health Service Grant H-1659 and a grant from the Albert and Mary Lasker Foundation.

young subjects and elderly male subjects and the 24-hour samples of the elderly female subjects did not undergo manual extraction with ethyl acetate but continuous extraction for eight hours under reduced pressure in ether-liquid type extractors (Ace glass). The internal temperature of the solvent receiving-distilling flask was maintained at 37 °C.

After the initial separation of the urinary extract by paper chromatography in the benzene-formamide system, the area of the paper chromatogram more polar than that containing THF and extending to the line of application was cut and eluted four times with methanol. The methanol eluate, concentrated under a stream of nitrogen, was then chromatographed in the chloroform-formamide system for forty-eight hours along with an adjacent strip to which THF had been applied as a standard compound. In the benzene-formamide system THF had a mobility approximately twice that of β-cortolone, which had a mobility approximately one and a half times that of cortolone. In the chloroform-formamide system the 20α and β epimers did not separate well from each other or from THF, but had much greater mobility (Rf value, 0.5 cm. per hour). The cortolones were identified by means of a glycerol test (6), periodic-acid oxidation in a solution of tetramethylammonium hydroxide, and detection of the resulting 17-keto-steroids (17-KS) with Zimmermann reagent, on a sample strip of the chloroform-formamide paper chromatogram. Usually a pronounced 17-KS reaction was obtained on the strip at a distance from the origin comparable to the distance traversed by the THF standard. The comparable area of the remaining chromatogram was cut and eluted with methanol. Aliquots were taken for quantitative determination of steroid glycerols by the periodic-acid oxidation method of Talbot and Eitingon (7) and the subsequent determination of the 17-KS product by the Zimmermann reaction using the Allen correction equation (8). The values for the oxidation product, 11-ketoetiocholanolone, were corrected by a factor of 84 per cent for molecular weight difference between it and its precursor, β-cortolone (see Addendum). Additional corrections were made for aliquots taken for Porter-Silber determinations prior to paper chromatography, for paper strips removed for testing, and for 20 per cent loss of material on the paper chromatograms. Another aliquot was removed for determination of sulfuric-acid chromogen spectra; the maxima obtained for β-cortolone were in the region 270–280, and 410 mμ.

RESULTS

Experiments to determine the recovery of cortolones added to urine were not undertaken owing to the scarcity of the pure compounds of both epimers. However, urine which had already been incubated with glucuronidase and extracted with ethyl acetate was re-incubated with glucuronidase for an additional twenty-four hours and re-extracted with ethyl acetate. This second incubation released less than 5 per cent of the amount of cortolone obtained from the first 24-hour incubation. Urine samples were also incubated with glucuronidase for twenty-four hours and extracted with ethyl acetate for 2 separate eight-hour periods. The second extraction led to an average recovery of 4.5 per cent additional cortolone.

In a preliminary experiment the cortolone eluates from the chloroform-formamide paper chromatograms for young and elderly subjects of a previous study were pooled, oxidized with periodic acid, and the oxidation product run on a paper chromatogram for separation of 17-KS. A metab-

olite producing a positive Zimmermann reaction and having a mobility similar to that of 11-ketoetiocholane-3-ol-17-one was obtained. This material was identified as 11-ketoetiocholane-3-ol-17-one by infrared analysis.

Periodic-acid oxidation by the method of Talbot and Eitingon was carried out on quadruplicate aliquots of 25, 50, 75, 100 and 150 micrograms (μg.) of Δ^4-pregnenetriol-17α,20α,21-one-3. The average recovery was 99 per cent of the theoretical yield, with a range of 95–113 per cent. Similar recoveries were obtained by the sodium-bismuthate method of Brooks and Norymberski (9). However the former method was chosen both for its specificity for glycerol side chains and for the fact that the urinary pigment which frequently accompanies the cortolone and which interferes with the Zimmermann reaction was removed to a large extent by the solvent washes used in this method.

Since the 20α and β-cortolone epimers did not separate well in the chloroform-formamide system, both compounds were measured by the method described. However, a pool was made of 20 eluates consisting of 60 per cent or more of each cortolone sample remaining after aliquots were removed for oxidation and determination of sulfuric-acid chromogens. A substantial amount of a crystalline substance was obtained from this pool. This substance was recrystallized and submitted for infrared analysis. Its spectrum was identical with that of β-cortolone. The melting point (M.P.) was 257–258.5° C., whereas that for the known compound has been reported as 260–261.5° (1) and 263–266° (10), respectively. The M.P. for the 20α epimer has been reported as 208–209° (1). The mother liquors were submitted to paper chromatography in the benzene-formamide system for five days, in attempts to separate any of the 20α epimer that might be present. The results were essentially negative. Additional attempts at such separations of individual cortolone eluates turned out similarly. No identifiable amounts of the 20α epimer were obtained. The method described appears to measure largely the β-cortolone present in urinary extract.

β-Cortolone and Porter-Silber (PS) values for the total urinary extract prior to chromatographic separation, and values for THF, ATHF and THE for the 10 young and 10 elderly subjects are presented in Table 1. The excretion of β-cortolone ranged from 0.3 to 2.9 mg. per twenty-four hours and was of the same order as that of ATHF; there was a decline in output in the elderly subjects, similar to that for THF, ATHF and THE. The young women excreted less than the young men. The values for THF, ATHF and THE were similar to those obtained previously for the respective sex or age group (5). In Table 2 are listed the mean values of the 4 metabolites expressed in terms of mg. per Gm. of creatinine excretion. An average of 0.8 mg. of β-cortolone per Gm. of creatinine was excreted by all subjects. The range of values for each age group suggested that in no

TABLE 1. MEAN EXCRETION VALUES OF TOTAL PS, THF, ATHF, THE AND β-CORTOLONE (MG./24 HRS.) FOR YOUNG AND ELDERLY MEN AND WOMEN

Subjects	Number	Median age (yrs.)	Creatinine (Gm./24 hrs.)	Total PS steroids	THF	ATHF	THE	THF, ATHF +THE	β-Cortolone	THF, ATHF THE +β-Cortolone
Young men	5	25 25–32*	1.46 0.64–1.93	6.9 3.5–8.7	2.2 0.8–2.8	1.4 0.6–3.1	4.3 1.0–6.0	7.9 2.5–11.8	1.9 0.3–2.9	9.8 2.9–14.7
Young women	5	23 20.29	1.40 0.85–1.79	5.1 2.5–7.6	1.9 0.5–3.7	1.3 0.5–1.6	3.0 1.8–4.5	6.2 2.8– 8.5	1.1 0.5–1.6	7.3 3.3– 9.9
Older men	5	76 72–82	1.08 0.83–1.26	3.7 2.4–5.0	1.5 1.0–2.2	0.8 0.3–1.2	1.6 0.9–2.5	3.9 2.7– 5.6	0.7 0.6–1.0	4.6 3.4– 6.1
Older women	5	82 81–87	0.70 0.46–1.00	2.4 1.5–2.9	1.0 0.6–1.3	0.5 0.3–0.8	1.5 0.9–2.0	3.0 2.0– 3.8	0.6 0.4–0.8	3.6 2.8– 4.0

* Range.

single group would the excretion of β-cortolone be significantly different from that in any other, but the excretion in young men was found to be generally greater. The values of THF, ATHF and THE per Gm. of creatinine for each age group were not significantly different from each other, as previously observed (5).

The ratios (32, 20 and 48 per cent) for excretion of THF, ATHF, and THE to their total excretion are presented in Table 3; they were similar to those previously obtained. THF and ATHF comprise approximately 50 per cent and THE the other 50 per cent of the total. In addition the mean ratios of excretion of β-cortolone to the total excretion of THF,

TABLE 2. MEAN EXCRETION VALUES OF TOTAL PS, THF, ATHF, THE AND β-CORTOLONE EXPRESSED AS MG. PER GM. OF CREATININE OUTPUT

Subjects	Number	Total PS steroids	THF	ATHF	THE	THF, ATHF +THE	β-Cortolone	THF, ATHF, THE +β-Cortolone
Young men	5	4.8 4.3–5.4	1.5 1.3–1.9	1.0 0.4–2.2	2.8 1.6–4.2	5.3 3.6–8.3	1.2 0.5–2.0	6.5 4.6–10.3
Young women	5	3.7 2.9–4.3	1.3 0.6–2.3	0.9 0.6–1.1	2.1 1.7–2.5	4.3 3.1–5.1	0.8 0.6–1.0	5.1 3.8– 5.8
Older men	5	3.5 2.4–4.8	1.4 0.8–2.7	0.8 0.2–1.2	1.6 0.8–3.0	3.8 2.1–6.7	0.6 0.5–0.8	4.4 2.7– 7.3
Older women	5	3.4 2.9–4.2	1.5 0.9–2.4	0.9 0.3–1.2	2.2 1.3–2.9	4.6 2.7–5.8	0.9 0.8–1.8	5.5 3.2– 6.8
Total subjects	20	3.9± .19 2.4–5.4	1.4± .12 0.6–2.7	0.9± .10 0.2–2.2	2.2± .19 0.8–4.2	4.5± .32 2.1–8.3	0.9± .09 0.5–2.0	5.4± .39 2.7–10.3
Total subjects*	60	3.7± .12 2.2–5.4	1.2± .07 0.3–2.7	0.8± .06 0.2–2.5	2.0± .09 0.8–4.2	4.0± .17 1.6–8.3		

* Total from this and previous study (5).

TABLE 3. THE MEAN RATIOS OF EXCRETION OF THF, ATHF, THE AND β-CORTOLONE TO TOTAL EXCRETION OF THF, ATHF AND THE

Subjects	Number	THF	ATHF	THE	β-Cortolone
		THF, ATHF+THE			
Young men	5	30.1 23–38	18.1 6–27	51.8 41–65	22.6 13–28
Young women	5	28.7 17–46	20.4 18–27	50.9 36–65	18.0 14–23
Older men	5	37.1 31–45	22.0 6–39	40.9 30–49	18.9 9–29
Older women	5	33.1 18–41	18.1 10–24	48.8 41–58	20.8 11–41
Total subjects	20	32.2±1.8 17–46	19.7±1.7 6–39	48.1±2.0 30–65	20.1±1.6 9–41
Total subjects*	60	30 ±1.0 17–46	19 ±1.0 6–39	51 ±1.3 30–65	

* Subjects from this and previous study (5).

ATHF and THE were calculated for each age group and for all subjects. The similarity of the ratios for each group (23, 18, 19 and 20 per cent) and the small standard error for all 20 subjects indicate that β-cortolone is also excreted in a proportionately constant manner. Correlation coefficients for the excretion of β-cortolone with THF, ATHF, THE, and the sum of the 3 metabolites were 0.70, 0.71, 0.92 and 0.91 respectively. All these values were statistically significant at the 1 per cent level of confidence. The highest value of r for β-cortolone was obtained with THE, which has a similar ketone configuration at position-11 of the steroid molecule.

DISCUSSION

Cope and Black (11), using orally administered cortisol-4-C^{14} and measuring the specific activity of urinary THE, found that the urinary excretion of cortisol metabolites ranged from 4.9 to 27.9 mg. per day in normal subjects. Petersen (12), also by means of specific activity measurements, estimated that THF, ATHF, THE and allo-THE account for approximately 50 per cent of cortisol metabolites. Since allo-THE apparently accounts for less than 1 per cent of these metabolites, our 24-hour excretion values for THF, ATHF and THE presumably should be approximately 50 per cent of those obtained by Cope and Black. The values

for our young normal subjects (2.5 to 11.8 mg. per twenty-four hours) when multiplied by a factor of 2 are of the same order as those obtained by these investigators.

Fukushima and associates (1) estimated that cortolone, β-cortolone, cortol, and β-cortol constitute a minimum of 30 per cent of the metabolites of cortisol, on the basis of isotopic dilution studies. The values we obtained for what appears to be essentially β-cortolone account for 9 to 11 per cent of the cortisol metabolites and one-third of the foregoing estimate for urinary cortolone and cortol; the other two-thirds should be in the form of cortols or 20α-cortolone. Areas of the paper chromatogram more polar than those from which β-cortolone was eluted did react positively to the test for glycerols. Sulfuric-acid spectra of eluates of some of these areas suggested the presence of cortols, but periodic-acid oxidations revealed that the amounts of additional metabolites with glycerol side-chains were small in comparison to the amounts of β-cortolone. The excretion of this family of steroids may possibly be less in normal subjects than in the subjects of Fukushima *et al.* However, it is conceivable that recoveries of cortols and cortolones from urine may be incomplete, or that losses may be incurred with the mild alkali and water washes of the ethyl-acetate extract. We recently obtained β-cortolone in amounts sufficient to conduct recovery experiments which are currently in progress. Further investigations are also being made to determine conclusively whether or not small amounts of 20α-cortolone are measured along with the larger amounts of 20β-cortolone.

The excretion of β-cortolone, as determined by the described method, conforms to a proportionately regular pattern. It declines with age when estimated in terms of mg. per twenty-four hours. When expressed in terms of mg. per Gm. of creatinine excretion, the difference due to age is not marked. The ratio of the excretion of β-cortolone to that of THF, ATHF and THE is relatively constant for both sexes and age groups. The highly significant correlation coefficients obtained for β-cortolone versus THF, ATHF and THE confirm a common origin.

Acknowledgments

The authors are indebted to Dr. D. K. Fukushima, Sloan-Kettering Institute for Cancer Research, New York City, N. Y. for 3α,17α,20β,21-tetrahydroxypregnane-11-one and its 20α epimer; to James Carlo for facilitating the collection of samples from elderly subjects; to Edgar Nichols for determination of infrared spectra; and to Norman Gibree for creatinine determinations.

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ADDENDUM

A series of recovery experiments of β-cortolone from urine, mentioned in the foregoing discussion have been completed. The authors are grateful to Dr. L. H. Sarrett (Merck & Co., Inc., Rahway, N. J.) for the additional amounts of β-cortolone which made these possible.

Preliminary experiments suggested that losses of β-cortolone occurred after the ethyl-acetate extraction of urine. Fukushima *et al.* (1) found evidence of cortolones and cortols in the "phenolic" fractions of the urinary extracts. We examined the 0.1 *N* NaOH and water washes of the ethyl-acetate extracts (3) for the presence of β-cortolone.

In 4 cases, 400-μg. samples of β-cortolone were added to distilled water in volumes comparable to 12-hour urine aliquots. The water containing the β-cortolone was continuously extracted by the usual procedure with ethyl acetate. The resulting extract was washed with alkali and water. The alkali washes were combined for pairs of samples and continuously extracted at pH 7.0 with ethyl acetate. The ethyl-acetate extracts of the alkali washes were concentrated and submitted to paper chromatography along with the washed ethyl-acetate extracts in both the benzene-formamide and chloroform-formamide systems. After detection and elution from the paper chromatograms the β-cortolone eluates were subjected to periodic-acid oxidation and subsequent Zimmermann determinations. The values were estimated in terms of aliquots of β-cortolone (10, 20 and

30 μg., respectively) taken from the stock solution at the time of addition of the 400 μg. to the water.

Subsequently, 400-μg. portions of β-cortolone were added to each of two 12-hour urine samples which were not subjected to glucuronidase hydrolysis. Each alkali wash was individually extracted. In addition, two 12-hour urine aliquots were incubated with glucuronidase and subjected to the same procedures. The results of these experiments are presented in Table 4.

The mean recovery of β-cortolone from the aqueous and urinary ethyl-acetate extracts was 60 per cent. The mean recovery from these extracts and extracts of the water and alkali washes was approximately 80 per cent. The recovery of 60 per cent

TABLE 4. RECOVERY OF β-CORTOLONE FROM AQUEOUS AND URINARY ETHYL-ACETATE EXTRACTS AND FROM ETHYL-ACETATE EXTRACTS OF ALKALI AND WATER WASHES

Experiment	Recovery of β-cortolone (μg.)*				Recovery of β-cortolone (%)			Ratio, NaOH + H_2O wash to ethyl-acetate ext.
	from ethyl-acetate extr.	from NaOH wash	from H_2O wash	total recovery	from ethyl-acetate extr.	from NaOH + H_2O wash	total recovery	
1. 400 μg. of β-cortolone + H_2O	242	46	3	291	60	12	72	20
2. 400 μg. of β-cortolone + H_2O	221	46	3	270	55	12	67	22
3. 400 μg. of β-cortolone + H_2O	266	72	0	338	67	18	85	27
4. 400 μg. of β-cortolone + H_2O	230	72	0	302	58	18	76	31
5. 400 μg. of β-cortolone + urine	256	49	39	344	64	22	86	34
6. 400 μg. of β-cortolone + urine	224	45	39	308	56	21	77	38
7. Urine + glucuronidase	732	242†	15	989				37
8. Urine + glucuronidase	707	241†	15	963				34
Mean recovery (%)					60.0	17.2	77.2	

* Expressed in β-cortolone equivalents.

† Substance exhibited an Rf value, a positive glycerol reaction and a positive Zimmermann reaction after periodic-acid oxidation similar to those shown by β-cortolone.

of added β-cortolone is in contrast to the 98–108 per cent recovery of equivalent amounts of THF and THE added to urine (3). Recoveries of quadruplicate 50-μg. samples of β-cortolone from paper chromatograms were 90 per cent or better. Since a correction is made for 20 per cent loss for such elutions, the unexplained 20 per cent of loss of β-cortolone was presumably not a result of paper chromatography. Periodic-acid oxidation and Zimmermann determinations were apparently not a source of loss, since 4 series of samples (10, 20 and 30 μg., respectively) of β-cortolone produced optical densities indicating 79–89 (average 83) per cent of equivalent concentrations of 11-ketoetiocholanolone. The theoretical yield of 11-ketoetiocholanolone from β-cortolone is 84 per cent. Thus 95–106 (average 99) per cent of expected 11-ketoetiocholanolone was obtained. Although the approximate 20 per cent loss of β-cortolone cannot be explained at this time, the recovery of approximately 60 per cent of β-cortolone added to water or urine seems reproducible. Therefore, the values given for β-cortolone both in the text and in the tables of this paper were corrected for a 40 per cent loss of β-cortolone which occurs as a result of the methods employed. The corrected values presumably more nearly approximate the values for β-cortolone in the urine of young and elderly male and female subjects.

THE PREPARATION OF AN APPARENTLY HOMOGENEOUS GONADOTROPIN FROM HUMAN MENOPAUSAL URINE*

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ABSTRACT

Extracts with gonad-stimulating activity, prepared from postmenopausal urine by the kaolin method, were purified on DEAE-cellulose columns. The resulting material was apparently a homogeneous glycoprotein, containing several N terminal amino-acids and possessing both follicle-stimulating and luteinizing activities.

THE extensive literature on human menopausal gonadotropin (HMG) includes only a few successful attempts at concentration of the activity. Johnsen (1) reported great concentration of activity achieved by a single pass through a Permutit column and subsequent ammonium-sulfate fractionation. Measurement of this concentration was based largely upon mouse uterine-weight assays. We have made extensive trials of this method and found it too capricious, both as to total recovery and concentration of activity, to serve as an extended basis for isolation. Dr. Johnsen was good enough to supply us with a sample of his material and we can confirm its high activity. However, it is chromatographically non-homogeneous. Rigas *et al.* (2) also reported purification of human gonadotropin, but the data are insufficient to permit appraisal.

Albert *et al.* (3) reported virtually a tenfold purification of their kaolin-acetone material. There was no loss of activity and the slope of the rat ovarian-weight dose-response curve remained unchanged. When prepared from postmenopausal urine and assayed by the rat ovarian-weight method, their material has sixty-one times the potency of HMG 20 A.

In our own work on urinary "tropic" hormones we found that alcohol precipitation yielded the best and most consistent recovery of activity. However, this procedure is not suitable for isolation studies because of

Received December 29, 1958.

* This study was supported in part by Grant No. A 381 (C4–5) from the National Institutes of Health, Bethesda, Maryland.

the huge volume which must be handled. We therefore developed the procedure described in this report.

METHOD

After some study and preliminary work, it was decided to use the kaolin method of Albert (4) for preparation of the initial concentrate. When adapted for processing 5-gallon lots of urine, it has yielded a consistent concentrate which is readily handled.

The initial concentrate can be further purified with little loss of activity if the wet acetone precipitate is extracted with 10 per cent ammonium acetate in 59 per cent ethanol. The gonad-stimulating activity is found in the soluble portion (which contains about 40 per cent of the solids) and can be precipitated by the further addition of ethanol to 80 per cent. This precipitate is dried under a stream of nitrogen and is designated as stage II HMG. It was employed in all of the studies to be outlined here.

Our previous successful experience with swine gonad-stimulating hormones led us to a trial of DEAE-cellulose columns for fractionation. The activity is readily absorbed on such columns and eluted with buffers. A 20-Gm. portion of stage II material is absorbed on a neutral 100-Gm. column of DEAE-cellulose. Results are most reproducible if the columns are handled as follows: Distilled water is run through until a pH of 7.0 is attained in the eluate. The stage II material is then made up in distilled water at a concentration of 1 per cent and run through the column. The columns are all jacketed, and chilled water is circulated through the jacket. About 1500 mg. of inactive material can be eluted from the column with 0.005 *M* phosphate buffer; if this step is omitted, the next step yields a poorer product. The buffer is then placed in a reservoir of 200 ml. capacity and a gradient is run through the reservoir with 0.5 *M* NaCl in 0.1 *M* NaH_2PO_4. This serves to elute a large amount of material. The first 400–500 ml. contains the bulk of the gonad-stimulating hormone activity and 10 to 20 per cent of the solids. There is some disparity in recovery by different assay methods—the greatest with regard to follicle-stimulating hormone (FSH) as measured by the method of Steelman and Pohley (5), and the least with regard to mouse uterine-weight activity. The eluted material is dialyzed in the cold room against repeated changes of distilled water until it is qualitatively salt-free (about 72–96 hours). The material is then lyophilized. Desalting with a mixed bed resin (MB3) has not been as successful as dialysis.

The material eluted with the foregoing gradient and assaying 20 per cent or more of Armour hog pituitary FSH (264-151-X) is combined from several runs, made up to 1 per cent in neutral distilled water and again placed on the column. Then 0.005 *M* phosphate buffer to a pH of 7.0 is run through

and a little inactive material eluted. A 200-ml. portion is placed in the reservoir and a gradient with 0.1 *M* NaH_2PO_4 started. After an initial period of elution, which yields material unhomogeneous by electrophoresis, a fraction is eluted which is apparently homogeneous.

PURITY AND ACTIVITY

This homogeneous fraction meets the following criteria for purity: 1) it exhibits a single sharp peak upon further DEAE-cellulose column absorption and elution; 2) it migrates as a single substance during paper electrophoresis at pH's of 6.8 and 8.6; 3) only a single peak is obtained when the material is ultracentrifuged; and 4) when analyzed in the ultracentrifuge by the Archibald method (6) it appears to have a molecular weight of 30,600. Table 1 shows the amino-acid composition of a typical sample of this material.

TABLE 1. AMINO-ACID COMPOSITION OF HUMAN URINARY FSH

Amino-acid	Per cent	Amino-acid	Per cent
Arginine	4.0	Threonine	4.4
Histidine	3.4	Leucine	5.1
Lysine	5.0	Isoleucine	2.7
Tyrosine	3.9	Valine	2.9
Phenylalanine	3.7	Glutamic acid	11.6
Cystine-Cysteine	4.1	Aspartic acid	6.3
Methionine	1.1	Glycine	2.8
Serine	2.6	Alanine	3.1
		Proline	2.7

The material approximates Armour FSH standard (264-151-X) when assayed by the method of Steelman and Pohley (5). It has only about 0.5 per cent of the stimulating activity of Armour LH standard (227-80) on the ventral prostate of the hypophysectomized male rat, and contains 6 mouse uterine-weight units per milligram.

In the process of purification no other major fractions with gonad-stimulating hormone activity are obtained.

Assay of the material for N terminal amino-acids by the DNP method, indicates the presence of several of these acids.

Since the cruder DEAE-cellulose eluates possess up to twice the ventral-prostate stimulating activity of this fraction, and since this fraction is the only one obtained on further purification which displays this activity, it appears that human urine, like animal pituitary tissue, contains material

which is not of itself active in this assay but which synergizes with gonadotropin to yield greater activity.

Acknowledgment

The authors wish to thank the nurses of the following New Orleans institutions for supervising the collection of the many gallons of urine used in this study from the residents of these institutions: St. Anna's Asylum, St. Margaret's Daughters Home, Fink Asylum and The Little Sisters of the Poor.

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SEPARATION OF HUMAN THYROTROPIN FROM PLASMA BY ION EXCHANGE CHROMATOGRAPHY*

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ABSTRACT

Thyrotropic activity was recovered completely from dialyzed mixtures of 7–35 ml. of human plasma and crude human pituitary preparations by chromatography on pH 7.2–7.8 Amberlite IRC-50 columns, eluted with a gradient to 2 *M* sodium chloride. This procedure was successful over a range of 0.08–17 U.S.P. milliunits per milliliter of plasma and separated thyrotropin from the nondialyzable substances in plasma interfering with the bioassay method used. Endogenous thyrotropic activity (0.2 milliunit per milliliter) was measured in the plasma of a myxedematous patient by this method. The active fraction was in the same location in the elution chromatogram as the activity recovered from mixtures of human plasma and crude human pituitary thyrotropin, or from the latter alone.

PREVIOUS studies of the level of endogenous thyrotropin in human blood have led to various results (1–10). The sensitivity of the bioassay has been a problem, and some preliminary procedure for concentration has usually been necessary. Several methods of assay have demonstrated either nonspecific responses (11–14) or interference by nonthyroidal substances in plasma (9) or urine (14, 15). Thus there is need for a specific method of recovery and purification that provides both concentration from dilute solution and separation from interfering substances.

Assay of thyrotropic activity by weight-response of bovine thyroid slices (16) is simple, specific, and usually sensitive to $\leq$0.03 U.S.P. milliunits per milliliter, but direct application to blood serum or plasma is precluded by the presence of some nondialyzable substances that interfere with the assay. Figure 1 illustrates this problem: A dose-response curve

Received January 16, 1959.

* Supported by U. S. Public Health Service Grant #A-701 (C4), the National Foundation for Infantile Paralysis, and the Veterans Administration.

A preliminary report of this work was presented December 16, 1958, at the Veterans Administration Annual Medical Research Conference, Memphis, Tenn.

† Veterans Administration Clinical Investigator. This work was begun during the tenure of a Fellowship of the National Foundation for Infantile Paralysis.

for a solution of crude human pituitary thyrotropin is compared with the corresponding curve for a dialyzed mixture of this preparation and human plasma; no activity is demonstrable in the mixture. However, chromatography on the carboxylic ion exchange resin, Amberlite IRC-50, which has afforded purification and complete recovery of thyrotropin from bovine (17, 18) or human (19) pituitary extracts, permitted recovery of the activity free of appreciable inhibitors.

Carboxymethylcellulose (20), which has also been satisfactory for chromatography of bovine (21) and human (19) pituitary thyrotropic preparations, proved less useful for separation of human thyrotropin from plasma.

MATERIALS AND METHODS

Reagents of analytical grade and distilled water were used throughout. All operations, except bioassay and preparation of reagents, were carried out at 0–5° C., with cold solutions. For all dialyses, Visking cellophane tubing ($\frac{5}{8}$-inch diameter) was used.

Crude human pituitary thyrotropin solution was prepared by the method described previously (19). The 2 batches used had potencies of about 18 and 11 milliunits per milliliter.

Venous blood was drawn into a 150-ml. vacuum bottle (Baxter) containing 1/10 the final volume (about $\frac{1}{5}$ the volume of plasma recovered) of an anticoagulant solution.[1] This solution consisted of 1 Gm. of tetrasodium ethylenediaminetetraacetate (Versene) per 100 ml., made isotonic in sodium chloride and containing 0.01 *M* propylthiouracil. The blood inflow rate was restricted to rapid dropping, just short of a thin stream, in order to minimize hemolysis. Plasma was separated promptly by centrifugation and was stored frozen in polyethylene vials. Plasma used for most recovery studies[1] was from a patient with panhypopituitarism ("PHP" in the tables); pooled "normal" plasma from 4 patients without clinical endocrine abnormalities ("PN" in the tables) was also used.

For studies of endogenous thyrotropin, the following refinements in procedure were made: The anticoagulant solution was adjusted to pH 7.2–8.0 before the blood was drawn; the vacuum bottle was kept in an ice-bath while the blood was drawn; and the packed cells were resuspended in isotonic sodium chloride solution equal in volume to the plasma recovered, and recentrifuged. The combined supernatant solution was then stored frozen, for subsequent chromatography.

Chromatography. Plasma was thawed at room temperature and mixed with the chosen volume of crude human pituitary thyrotropin. This mixture was diluted to 50–100 ml. with isotonic sodium chloride and dialyzed for twenty-four to forty-eight hours against 2000 ml. of 0.005 *M* sodium phosphate buffer (pH 7.6) containing 0.001 *M* propylthiouracil. Continuous stirring was maintained in the dialyzing buffer, which was changed three times. The dialyzed suspension was centrifuged and the precipitate resuspended in 20–50 ml. of the same buffer and recentrifuged.

The combined supernatant solution, diluted to 100–150 ml. with the same 0.005 *M* buffer, was passed through the 2×20 cm., pH 7.2–7.8 column of 200–400 mesh Amberlite IRC-50 and washed with 9–14 ml. of the same buffer per ml. of plasma. Elution was

[1] Plasma volumes recorded in this article were corrected for the volume of anticoagulant solution.

accomplished with a gradient to 2 *M* sodium chloride, using a constant-volume mixing chamber originally containing 250 ml. of the described 0.005 *M* buffer. Fractions of 20 ml. were collected. Flow was by gravity, and the rate was kept below 30 ml. per hour (below 1 drop every ten seconds). Each receiving vessel contained 5 ml. of 0.5 *M* sodium chloride in a sodium barbital buffer of pH 8.5, 0.4 ml. of 0.05 *M* propylthiouracil, and 0.2 ml. of a 2.5 mg./ml. solution of human serum albumin, as "stabilizers" for the 20-ml. eluate fraction (19).

The fractions were dialyzed for twenty-four to forty-eight hours against 3000 ml. of the Krebs-Ringer phosphate medium containing 0.001 *M* propylthiouracil used in the assay (16). Continuous stirring was maintained in the medium, which was changed three times. Aliquots of each fraction were stored frozen; no sample was subjected to more than one freezing and thawing.

Bioassay. Thyrotropic activity was determined by the previously described (16) method, utilizing the weight-response of fresh bovine thyroid slices incubated twenty-one hours at 37° C. in Krebs-Ringer phosphate medium. Groups of 5 (usually) to 10 were used, and each sample was tested at two to three doses at threefold to fourfold intervals. Statistical procedures were as described before (16); 95 per cent ($P=0.05$) confidence limits according to the method of Bliss (22, 23) are included (in parentheses) after the mean values. The initial crude human pituitary thyrotropin preparation was used as the concurrent standard in the assay of fractions obtained in recovery experiments.[2] Primary U.S.P. standard thyrotopin was used in the assay of endogenous thyrotropin.

RESULTS

Recovery experiments

As illustrated in Figure 1 (V_5 and V_6), the active eluate fractions showed log dose-response slopes which were parallel, within statistical requirements, to that of the standard. The results are summarized in Table 1. There was no detectable endogenous activity in the plasmas used. In 14 out of 15 experiments there was 100 per cent recovery, within the limits of assay error.[3] Without the single outlying high value, the data could be combined by the method of Bliss (24), yielding a mean recovery of 105 (93–120) per cent. Over the range 0.08 to 17 milliunits per milliliter of plasma, no trends in percentage recovery were noted with the various amounts of crude human pituitary thyrotropin or plasma. No significant differences in percentage recovery were found among combined values calculated (24) for the following groups: 7–12 ml. and 32–35 ml. of plasma; and 0.25, 0.5–0.75, 1.0–2.0, and 7.0–7.6 ml. of crude human pituitary thyrotropin. Table 2 shows the results of 5 experiments, in each of which two columns were run simultaneously, differing in only one factor. Again, no differences in percentage recovery were noted between 0.5 and 2.0 ml. of crude human thyrotropin, 12 and 35 ml. of plasma, 2×20 cm. and 4×25 cm. column

[2] See discussion in reference 19.

[3] One out of 20 would be expected to be beyond the 95 per cent confidence limits, but the single high "outlying" value may well have been due to a technical error.

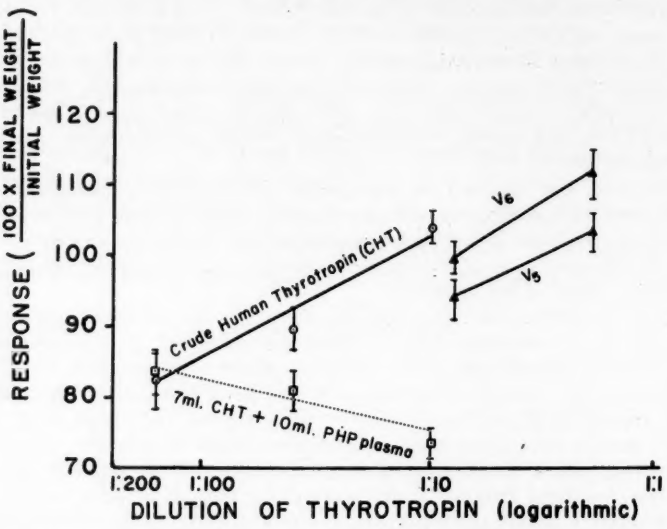

FIG. 1. Interference by plasma with the assay of thyrotropic activity; recovery of active fractions (V_5 and V_6) by chromatography on Amberlite IRC-50. The mixture of plasma and crude human (pituitary) thyrotropin solution was dialyzed two days prior to assay of an aliquot and chromatography of the remainder (see text under "Methods"). (PHP = panhypopituitary.)

size, or pH 7.2–7.3 and 7.7–7.8. In contrast, reducing the volume of the post-adsorption run wash well below the usual 9–14 ml. per ml. of plasma[4] resulted in incomplete recovery.

Partial breakthrough of activity in the effluent from the adsorption run was observed in an experiment using an IRC-50 column at pH 8.1–8.9.

In all these elution chromatograms, activity appeared in the same location as it did with crude human pituitary thyrotropin alone (19). In each experiment, activity was limited to one or two 20-ml. fractions, collected between 60 ml. and 140 ml. from the start of the gradient. In the majority of experiments using less than 2.0 ml. of crude thyrotropin, activity was limited to one 20-ml. fraction. Most of the activity usually preceded, by one eluate fraction, the main visible (tan or yellow) protein band, which was also confined to one or two fractions. In 5 experiments there was complete separation of activity and color. In only 3 experiments did the main

[4] This proportion of wash was originally chosen by studying the optical density at 280 mμ of column effluents in experiments in which propylthiouracil (which absorbs at that wavelength) was omitted from the reagents. Most of the total plasma protein is washed through the column in the pH range used.

TABLE 1. CHROMATOGRAPHY OF MIXTURES OF CRUDE HUMAN PITUITARY THYROTROPIN AND PLASMA ON AMBERLITE IRC-50

Vol. of crude human pituitary thyrotropin (ml.)	Vol. (ml.) and source of plasma	Per cent recovery (95% confidence limits)
0	32 PHP*	Not detectable†
	34 PN*	Not detectable‡
0.25§	7 PHP	356 (225–543)
	12 PHP	117 (82–166)
	32 PN	71 (44–116)
	32 PN	100 (68–140)
	32 PN	100 (64–148)
	35 PHP	133 (94–189)
0.5§	7 PHP	73 (47–113)
	8 PHP	123 (68–240)
0.75§	32 PN	119 (67–202)
	32 PN	162 (92–272)
1.0§	7 PHP	153 (98–253)
2.0§	7 PHP	90 (55–145)
	8 PHP	77 (35–169)
7.0‖	8 PHP	123 (65–233)
7.6‖	8 PHP	74 (44–123)

* Abbreviations: PHP: panhypopituitary.
PN: pooled normal.

† <0.06 ml. crude human pituitary thyrotropin (<0.02 U.S.P. milliunit per milliliter of plasma).

‡ <0.05 milliunit per milliliter.

§ Potency about 11 milliunits per milliliter.

‖ Potency about 18 milliunits per milliliter.

activity and color coincide, and in no case did activity lag behind color in the elution chromatogram.

Carboxymethylcellulose was also used in preliminary experiments, for chromatography of mixtures of human plasma and crude human pituitary thyrotropin, in a procedure similar to that described for IRC-50. With pH 6.0–7.0 columns, no breakthrough of activity in the effluents from adsorption runs was found. Activity was eluted with a gradient to 1 *M* or 2 *M* sodium chloride, but it was spread so widely (four to ten 20-ml. fractions)

TABLE 2. STUDY OF 5 FACTORS IN DUPLICATE IRC-50 CHROMATOGRAPHY EXPERIMENTS

Vol. of crude human pituitary thyrotropin (ml.)	Vol. of plasma (ml.)	pH	Column size (cm.)	Vol. of post-adsorption run wash (ml.)	Per cent recovery (95% confidence limits)
0.5	7	7.4	2×20	100	73 (47–113)
2.0	7	7.4	2×20	100	90 (55–145)
0.25	12	7.4–7.5	2×20	125	117 (82–166)
0.25	35	7.4–7.5	2×20	300	133 (94–189)
0.25	32	7.2–7.3	2×20	300	100 (68–140)
0.25	32	7.7–7.8	2×20	300	100 (64–148)
0.75	32	7.2–7.3	2×20	300	119 (67–202)
0.75	32	7.2–7.3	4×25	300	162 (92–272)
0.25	32	7.6–7.7	2×20	300	71 (44–116)
0.25	32	7.6–7.7	2×20	175	32 (9– 75) reassay: <50

as to make the procedure useless. At pH 7.3–7.4 there was partial breakthrough—12 (range, 5 to 20).

Application to endogenous human thyrotropin: preliminary report

Two other control experiments provided further evidence of applicability to endogenous thyrotropin in plasma. To rule out an effect of Versene, we mixed 4 ml. of crude human pituitary thyrotropin solution, 35 ml. of isotonic sodium chloride, 5 ml. of the anticoagulant solution (described previously), and 5 ml. of 0.5 *M* sodium phosphate buffer (final pH 7.4) and compared this solution with a mixture of 4 ml. of the thyrotropin solution, 40 ml. of isotonic sodium chloride, and 5 ml. of 0.5 *M* sodium phosphate buffer (final pH 7.2). After both mixtures stood overnight at 0–5° C., assay revealed no difference in potency. Also, 1 ml. of crude human pituitary thyrotropin solution was added to 33 ml. of blood (including anticoagulant) before letting it stand for fifteen minutes and processing it in the usual way. Recovery of activity was satisfactory, *i.e.*, 66 (range, 40–110).

Using the described method of chromatography on an IRC-50 column, we found endogenous activity of 0.21 (0.14–0.31) milliunits per milliliter in the plasma of 1 patient (A.H.) with myxedema of recent onset. Here again, the active fraction (60–80 ml. from the start of the gradient) was in the same place in the chromatogram as that occupied by the mixtures of hu-

man plasma and crude human pituitary thyrotropin or by the latter alone (19); also, the activity preceded the main colored band by one fraction.

DISCUSSION

Previous workers have used serum for the study of endogenous human thyrotropin in blood. However, because of the ease with which thyrotropin is adsorbed on various substances (17), we used plasma routinely in order to avoid possible loss on the clot.

Chromatography on Amberlite IRC-50 apparently affords complete recovery of added human pituitary thyrotropin from plasma through a fairly specific method of good resolving power. Separation from nondialyzable substances interfering with the assay response was demonstrated regularly, even when great difficulty would be expected, *e.g.*, at minimal detectable levels of thyrotropin in about 35 ml. of plasma. On the other hand, the maximum ability of the procedure to concentrate thyrotropin was not tested, because of a feature of the *in vitro* assay method used. This feature was the requirement of 2–3 ml. of solution per beaker and thus the necessity for using about 20 ml. of the highest concentration for quantitative assay of a preparation. Hence, for convenience, 20-ml. eluate fractions were collected and, because half of each fraction was saved for possible repeated assay, the highest dose tested was a 1:2 dilution. Since in most experiments using less than 2 ml. (*i.e.*, less than about 22 milliunits) of added crude human pituitary thyrotropin per column the activity was confined to one 20-ml. fraction, it is reasonable to believe that chromatography of endogenous thyrotropin in plasma might also localize all activity in under 20 milliliters if smaller fractions were cut. This greater concentration would be useful with other assay methods (10) using 1 ml. or less per animal. Cutting smaller fractions would also probably permit greater purification of thyrotropin, but this does not seem necessary for quantitative assay. In any case, the observation that the activity usually precedes the main visible protein band in the elution chromatogram by one 20-ml. fraction, and the activity never lags behind the color, is a great practical convenience. For example, in the gradient elution of a 4×25 cm. column, the activity emerged 200 ml. behind that from the 2×20 cm. column, but in each case the usual relationship of activity to the visible protein color pertained.

The conditions reported here for chromatographic separation of human thyrotropin from plasma were similar to those found suitable for crude bovine or human pituitary thyrotropic preparations alone (17–19). Similarly, attempted adsorption of plasma plus crude human pituitary thyrotropin solution on Amberlite IRC-50 at pH 8.1–8.9 resulted in partial breakthrough of activity, as in the case of bovine pituitary preparations

(17). Moreover, the location of activity in the elution chromatogram was the same when recovered from crude human pituitary thyrotropin solution alone (19), mixtures of this solution plus plasma, or plasma from a myxedematous patient. These facts suggest that free human thyrotropin, presumably in cationic form, is the operative substance in this chromatographic procedure. It will be interesting to compare the endogenous levels of thyrotropin measured by the present chromatographic procedure with those found by application of a less specific separation method and assay in an intact animal, which may be capable of freeing thyrotropin from the bound form.[5] Full results on patients with and without clinical endocrine abnormalities will be presented in a subsequent article.

In the preliminary experiments with carboxymethylcellulose, partial breakthrough of activity in the effluent from the adsorption run was found at pH 7.3–7.4. Hence the pattern of adsorption, but not elution, on this adsorbent was similar to that found with crude human pituitary thyrotropin alone (19).

The fact that the "breakthrough" activity in these cases showed the usual log dose-response slope in assay implies that the interfering substances were adsorbed to the column. Presumably the gradient elution procedure permitted the separation of thyrotropin from interfering substances. We have not yet attempted to isolate these inhibitors.

Acknowledgment

The technical assistance of Florence Arnett, Julianne Henrie, Patricia Larson, Wilford MacFadden, and James Page is gratefully acknowledged.

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[5] Smelser (25) found that the chick could respond to the thyrotropic activity in an insoluble flavianic-acid precipitate from a crude pituitary preparation, but it is probable that the thyrotropin was merely co-precipitated (adsorbed) on the protein precipitate rather than bound in the chemical sense (17, 26).

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Letters to the Editor

THE EFFECTS OF HYPOPHYSECTOMY ON CERTAIN PARAMETERS OF THYROID FUNCTION IN TWO PATIENTS WITH GRAVES' DISEASE

TO THE EDITOR:

The effects of hypophysectomy on thyroid function in animals have been studied by many investigators. An immediate reduction in the ability of the thyroid to trap and bind iodine has been reported (1), along with a decreased rate of release of iodine from the gland (2) and a reduction in the level of plasma protein-bound iodine (3). Chromatógraphic studies in animals have shown a quantitative change in the pattern of iodine compounds identified in the plasma following hypophysectomy (4). As a result of these and other animal studies, it has been suggested (5) that autonomous regulation of the thyroid is of relatively little importance in the control of normal thyroid function.

The studies of Li and his associates (6) indicate that similar events take place in man. Following hypophysectomy of presumably euthyroid patients with metastatic breast disease, they found that the concentration of serum protein-bound iodine fell to a value of less than 3.0 μg. per 100 ml. within two to three weeks, and the thyroidal radioiodine uptake fell below 10 per cent within two to four weeks in almost all cases. The onset of clinical hypothyroidism was noted within four to six weeks following operation in most patients.

The behavior of the thyroid in patients with Graves' disease following hypophysectomy is of considerable interest. Since there is no state in the experimental animal analogous to Graves' disease in man, it has not been possible to examine the consequences of hypophysectomy in animals with this condition. We have had the opportunity to study 2 patients with Graves' disease following surgical hypophysectomy.[1] The preliminary findings are the subject of this communication.

CASE REPORTS

H.G. This 57-year-old white woman first showed the classic symptoms of Graves' disease four years before admission. Progressive infiltrative eye disease had been present

[1] We are indebted to Dr. Bronson S. Ray, Chief, Department of Neurosurgery, The New York Hospital—Cornell Medical Center, for this opportunity.

for one year. Previous therapy, which included antithyroid drugs and 2 therapeutic doses of radioactive iodine, had resulted in only partial control. Desiccated thyroid, triiodothyronine, ACTH, prednisone, cortisone, and finally lateral decompression were all utilized in an unsuccessful attempt to ameliorate the severe eye disease. Because of increasing proptosis, decreasing visual acuity, and increasing field defects, it was decided to perform hypophysectomy in an attempt to prevent progression of the ocular process. Within four weeks following total surgical hypophysectomy, there was a remarkable improvement in visual acuity and field defects, together with clearing of chemosis and infiltration. There was also a decrease in the size of the diffusely enlarged thyroid. Laboratory data are outlined in Table 1.

S.M. This 36-year-old woman had undergone a left radical mastectomy for carcinoma of the breast fourteen months prior to admission. She subsequently complained of increasing sacral pain, with radiation down both legs. A bilateral oophorectomy had been performed eleven months later. At the time of her admission to The New York Hospital there was evidence of liver and bone metastases.

Five years before admission the patient had been told of an enlarged thyroid gland and an elevated basal metabolic rate. She had subsequently taken various doses of propylthiouracil intermittently, the last course beginning three months prior to entry. The patient was admitted for hypophysectomy for palliation of metastatic breast carcinoma and was thought to be clinically thyrotoxic. There were no signs of ocular involvement. Laboratory data are outlined in Table 2.

COMMENT

Both patients experienced relief of the symptoms of hyperthyroidism following operation. Persistence of elevated thyroidal radioiodine uptakes was the most striking laboratory finding. Failure of the serum protein-

TABLE 1. LABORATORY DATA—PATIENT H.G.

Time* (days)	Thyroidal I^{131} uptake (%)	Serum PBI (μg./100 ml.)	Serum total iodine (μg./100 ml.)	BMR (%)	Serum cholesterol (mg./100 ml.)
− 18		8.7	9.4	− 5	187
− 1	57	9.5	9.5		
0			*Hypophysectomy*†		
+ 11	24	6.0	7.2	−26	
+ 21		6.0	6.6		235
+ 41	43			−17	186
+ 45		6.8	7.3	−20	
+ 59		7.8	8.6		
+ 89	49	5.5	6.3	−20	203
+178	30	5.6	5.8	−38	264

* Measured from day of operation.

† Medications: Cortisone, from day of operation. Triiodothyronine, from day 55 to day 164.

bound iodine (PBI) level to fall sharply was surprising. In Patient H.G., large doses of triiodothyronine either before or after hypophysectomy, failed to suppress thyroidal I^{131} uptake. This patient became clinically hypothyroid postoperatively and the symptoms of hypothyroidism responded poorly to replacement therapy with triiodothyronine.

It is possible that the first preoperative elevated I^{131} uptake in Patient S.M. was a "rebound" phenomenon following discontinuance of propylthiouracil, but repetition of the studies eight days later showed persistent

TABLE 2. LABORATORY DATA—PATIENT S.M.

Time* (days)	Thyroidal I^{131} uptake (%)	Serum PBI (μg./100 ml.)	Serum total iodine (μg./100 ml.)	BMR (%)	Serum cholesterol (mg./100 ml.)
− 9	96	4.6	4.6	+25	228
− 1	95	6.3	6.4	+ 9	197
0			*Hypophysectomy*†		
+ 4		3.5	3.9		
+12	83	4.5	4.7	+ 2	179
+40	68	8.9	9.0	− 3	174
+47					147

* Measured from day of operation.

† Medications: Propylthiouracil, until 18 days before operation. Cortisone, from day of operation.

elevation. Chromatography of the serum of both patients (reported elsewhere in detail (7)) showed the presence of thyroxine and triiodothyronine in virtually normal proportions after hypophysectomy.

The first explanation that suggests itself for the failure of a sharp fall in thyroidal I^{131} uptake and serum PBI level is that the operation was incomplete and some pituitary tissue remained. Criteria for completeness of hypophysectomy are difficult to define. In both patients urinary gonadotropin after operation decreased to a value below 6.5 m.u. per twenty-four hours. Patient H.G. was subsequently rehospitalized and her maintenance steroid therapy was withdrawn. No 17-hydroxycorticoids could be detected in the plasma, and urinary excretions of 17-ketosteroids and 17-hydroxysteroids were less than 2 mg. per twenty-four hours. The hypophysectomies in both these patients were performed by the surgeon who performed the operation in Li's series, so the technique was identical and both groups of patients may be readily compared from this point of view.

It is of interest that short-term (six-week) observations in a patient with

acromegaly who was hypophysectomized for treatment of this condition showed a similar failure of the thyroidal I^{131} uptake and serum PBI level to decrease from preoperative levels despite the development of clinical hypothyroidism.

Four recent clinical studies indicate that thyroid hyperfunction may occur in patients with little or no functioning pituitary tissue. Fajans (8) reported a patient in whom hyperthyroidism developed following twenty years of hypopituitarism induced by postpartum necrosis of the pituitary. Werner and Stewart (9) observed hyperthyroidism in a patient with a pituitary chromophobe adenoma and a fragment of normal pituitary. Albeaux-Fernet *et al.* (10) and McCullagh *et al.* (11) reported cases in which there was no fall in the serum PBI level following pituitary surgery. In the cases of McCullagh *et al.* an elevated thyroidal radioiodine uptake persisted following section of the stalk or cauterization of the anterior lobe. It is of interest that in both groups there was improvement in the infiltrative ophthalmopathy (as in our Patient H.G.) after operation.

The results of this study suggest that, following hypophysectomy in man, 2 common parameters of thyroid function are not uniformly affected, as might be expected. In 2 patients with Graves' disease who were hypophysectomized, neither the thyroidal I^{131} uptake nor the concentration of serum PBI fell to the hypothyroid level.

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CONGENITAL ADRENAL HYPERPLASIA AND PRECOCIOUS GONADOTROPIN SECRETION IN A 6-YEAR-OLD GIRL

To the Editor:

The purpose of this report is to describe a 6-year-old girl with congenital adrenal hyperplasia, fully mature Graafian follicles, and precocious secretion of gonadotropin.

METHODS

Urinary 17-ketosteroids were measured according to the method of Callow *et al.* (1). Technical difficulties, especially the lack of a spectrophotometer, prevented us from measuring pregnanediol and pregnanetriol. Hence "pregnanediol" is used to designate the material obtained in applying the method of Henderson *et al.* (2) for the estimation of pregnanediol. Urinary estrogens were estimated by the method of Jayle *et al.* (3). Urinary gonadotropin was extracted by the method of Albert (4) and the quantity of gonadotropin was measured in intact immature female mice according to the procedure of Klinefelter *et al.* (5). The adrenals were visualized by presacral air insufflation according to the technique of Ruiz Rivas (6).

CASE REPORT

V.D., born in 1951, was an only child. The mother experienced her first menstrual bleeding at the age of 15. At birth, the child had a slightly enlarged clitoris and no vaginal opening. In the postnatal period there were no symptoms of hypoadrenocorticism. At the age of 5 years, the girl was gaining height fairly rapidly. Pubic hair appeared and the clitoris increased in size.

She was first seen by us at the age of 6 years, when her height was 116 cm. Both

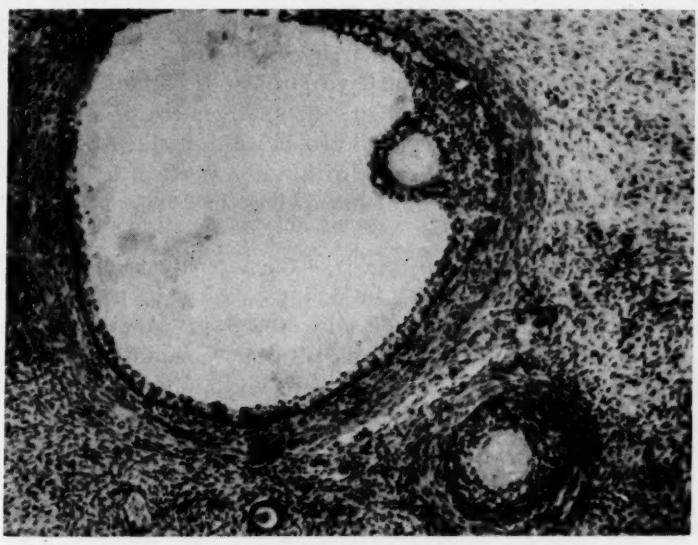

FIG. 1. Histologic section from the ovary of Patient V. D.
A well developed Graafian follicle is demonstrated.

height age and bone age were at the 7-year level. Pubic hair was scant. The clitoris was 2 cm. long. The orifice of the urethra was at the base of the clitoris and there was no vaginal opening. The breasts were not developed.

Urinary 24-hour excretions of steroids were as follows: 17-ketosteroids, 12.5 and 17.2 mg.; "pregnanediol," 30, 17 and 24 mg.; and estrogens, 25 and 24 μg. Serum electrolyte levels were normal. The eosinophil leukocyte count was 510 per cu. mm.; four hours after intramuscular administration of 25 I.U. of adrenocorticotropin (Prolek) the eosinophil count was 780 per cu. mm.—an increase of 53 per cent. Urinary gonadotropin excretion was 13 mouse uterine units per twenty-four hours. X-ray examination showed that both adrenals were enlarged.

Exploratory laparotomy. The uterus and tubes were of normal appearance and corresponded in size to those of an infant. The ovaries were larger than would be expected in a child 6 years of age. The ovarian capsule was thin. Bilateral biopsy of the ovaries was performed. Histologic examination showed follicles in various stages of development, from the primordial up to the Graafian form (Fig. 1).

DISCUSSION

On the basis of the case history and laboratory findings, the diagnosis of congenital hyperplasia of the adrenal cortex seemed clearly established. Impaired synthesis of glucocorticoids was compensated for, as there were no clinical signs of hypoadrenocorticism. The initial phase of hypersecretion of androgenic hormones from the adrenal cortex apparently took place in the fetal period. Up to her fifth year of age the girl presented no clinical

manifestations of hypersecretion of androgens; she was gaining in height at a normal rate, her clitoris did not enlarge, and there was no development of pubic hair. It was only in her fifth year that her parents noticed rapid growth, the appearance of pubic hair, and enlargement of the clitoris.

It is interesting that urinary gonadotropin was found. Brown also found gonadotropin in the urine of 2 girls with adrenocortical hyperplasia (7). Another striking feature of the present case was the development of Graafian follicles.

Patients with congenital hyperplasia of the adrenal cortex produce large amounts of estrogens. According to Wilkins *et al.* (8), the high levels of estrogens and androgens from the adrenal cortex cause amenorrhea in such patients, when adult, by inhibiting pituitary gonadotropin. The secretion of gonadotropin in the present case was not inhibited, presumably because of a relatively low level of estrogen.

It could be assumed that accelerated body growth, appearance of pubic hair and enlargement of the clitoris at the age of 5 years were connected with the precocious secretion of gonadotropin, *i.e.*, that the adrenogenital syndrome was joined with precocious puberty. Thus the case would present 2 anomalies, namely, congenital adrenal hyperplasia and precocious secretion of hypophyseal gonadotropin. The mammary glands were not developed, presumably because of the inhibition exerted by the increased androgenic hormones.

During her stay in the hospital, the girl was given cortisone acetate, 100 mg. every other day, for a month. This treatment resulted in a reduction of the 24-hour excretion of 17-ketosteroids to 3.5 and 2.8 mg., and of "pregnanediol" to 1.2 and 0.9 mg. Unfortunately, cortisone therapy was stopped after discharge from the hospital because the parents could not afford continued medication. It is to be regretted that we were not able to observe the effect of cortisone on breast development.

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EFFECT OF CHORIONIC GONADOTROPIN ON URINARY EXCRETION OF 17-KETOSTEROIDS IN A 10-YEAR-OLD GIRL WITH CONGENITAL ADRENAL HYPERPLASIA

To the Editor:

The effect of chorionic gonadotropin on urinary 17-ketosteroids in a 10-year-old girl with congenital adrenal hyperplasia is the subject of this communication.

CASE REPORT

N.S. had an enlarged clitoris at the time of birth in 1945. The vagina was not developed. At the age of 7 years the clitoris showed a further increase in size. Pubic hair appeared in the eighth year. Height increased rapidly after the seventh year. When she was seen by us in her tenth year, her height was 144 cm.; her height age was 12 years, and bone age 14 years. The 24-hour urinary excretion of steroids was as follows: 17-ketosteroids, 24.2 mg.; pregnanediol, 37.5 mg.; estrogens, 62.6 μg.; and free reducing corticosteroids, 1.2 mg. The levels of serum electrolytes were normal. Urinary gonadotropin excretion was less than 6.5 mouse uterine units per twenty-four hours. (References to laboratory procedures are given in the preceding paper.)

Exploratory laparotomy showed small but normal ovaries, tubes and uterus.

Special studies (*Fig. 1*). In the control period (July 25–September 3, 1955) the patient had been excreting an average of 25 mg. of 17-ketosteroids per twenty-four hours.

She was then given chorionic gonadotropin (Choriogonadin, Prolek) in a dosage of 3000 I.U. daily, for nine days. The excretion of 17-ketosteroids gradually increased until it reached 89 mg. per twenty-four hours on the ninth day of the study. There was no febrile reaction.

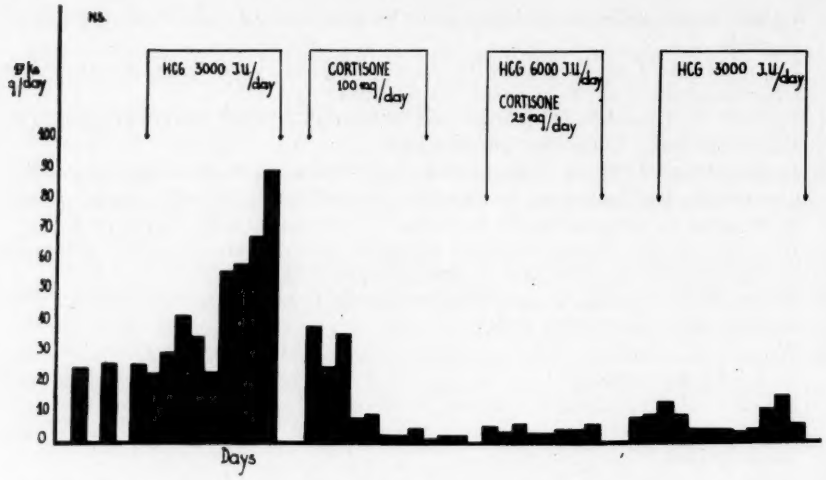

FIG. 1. Course of excretion of urinary 17-ketosteroids in N.S. during administration of chorionic gonadotropin (HCG) and cortisone acetate. The columns represent values for 24-hour excretion of 17-ketosteroids. (See text for details of the 4 study periods.)

Closely following the course of chorionic gonadotropin, cortisone acetate was given in a dosage of 100 mg. daily, for eight days. Urinary 17-ketosteroids dropped from 38.1 mg. to 4.4 mg. per twenty-four hours. (Later the girl was given 100 mg. of cortisone acetate every fourth day for a period of six and a half months.)

While in the phase of reduced secretion of adrenocorticotropic hormone induced by cortisone, the patient was again given chorionic gonadotropin (6000 I.U. daily, for eight days) along with cortisone acetate (25 mg. daily). There was no effect upon urinary 17-ketosteroids, which ranged from 3.0 to 5.9 (average 4.3) mg. per twenty-four hours.

Administration of cortisone was discontinued so as to allow for another rise in urinary 17-ketosteroids upon repeating the first part of the study. However, even as late as five months afterwards, 17-ketosteroid values were relatively low—4 mg. to 9 mg. per twenty-four hours. Five months after cortisone had been discontinued, chorionic gonadotropin was given in a dosage of 3000 I.U. daily, for nine days. Urinary 17-ketosteroid excretion ranged from 3.3 to 15.6 (average 7.5) mg. per twenty-four hours.

DISCUSSION

Unexpectedly, a rise in urinary 17-ketosteroid excretion was observed during administration of chorionic gonadotropin in a 10-year-old girl with congenital adrenal hyperplasia, prior to suppression of the adrenal cortex with cortisone. However, after adrenal suppression with cortisone, chorionic gonadotropin therapy produced no rise in urinary 17-ketosteroid excretion. Similarly, there was no rise when chorionic gonadotropin was administered several months later, after cortisone had been discontinued. In the latter period, the excretion of 17-ketosteroids remained within normal limits, even without cortisone therapy.

It could be assumed that chorionic gonadotropin raised urinary 17-ketosteroid excretion by stimulating the ovarian hilus cells (1). Were this so, the 17-ketosteroids should also be increased during suppression of the adrenal cortex, in contrast to our observations.

There remains another explanation for the rise in urinary 17-ketosteroids before adrenal suppression by means of cortisone. Chorionic gonadotropin may accelerate 17-ketosteroid excretion when secretion of adrenocorticotropic hormone is increased or normal, since in agonadal women with normal function of the adrenals there is no rise in urinary 17-ketosteroids during administration of chorionic gonadotropin (2, 3). Thus, the acceleration of androgenic manifestations near puberty, as seen in this case, may be due to the liberation of LH. This situation may parallel that in the fetus when both increased fetal adrenocorticotropin and high levels of maternal chorionic gonadotropin are present.

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URINARY 17-KETOSTEROIDS IN SURGICAL STRESS*

TO THE EDITOR:

The endocrinology of surgical stress has received much attention in the literature. Several excellent studies have contributed to our knowledge of steroid metabolism, during and following operation (1–3). It appears that

* This study was supported by a grant from the Josiah Macy, Jr. Foundation, aided by a contract between the Office of Naval Research, Department of the Navy and Yale University School of Medicine, NR 105057, and in part by a research grant (A 1587) from the National Institute of Arthritis and Metabolic Diseases, Public Health Service.

the measurement of 17-hydroxycorticoids in either plasma or urine constitutes a reliable evaluation of adrenal response to stress. On the other hand, the total excretion of urinary 17-ketosteroids seems to be less affected by surgical trauma. The introduction of accurate methods for the determination of the various 17-ketosteroid fractions, particularly enzyme hydrolysis, chromatography and identification of the isolated compounds by infrared analysis, makes it desirable to re-evaluate some of these data. Such an undertaking was carried out in the framework of a major metabolic study conducted by the Departments of Surgery, Medicine, and Obstetrics and Gynecology, Yale School of Medicine, of which this communication constitutes a preliminary report.

MATERIAL AND METHODS

The 4 patients of this study were housed in a metabolic unit. They were under constant supervision with carefully controlled intake and output. Urine was collected in 72-hour and 48-hour lots throughout the study. Steroid determinations were carried out in the preoperative period, on the two days following surgery, and once more after three to six weeks. In addition, 1 patient was studied following thirty-six hours of complete fasting during the preoperative control period. The operation performed on each patient is stated subsequently.

For comparison, 5 apparently healthy young males (medical students) were studied. Each was given 25 mg. of adrenocorticotropin (ACTH) administered by intravenous infusion in 5 per cent dextrose in water during a period of five hours. Individual baseline values were obtained from 48-hour urine pools and from 24-hour collections on the day of the infusion.

The chemical method for the determination of 17-ketosteroids has been described in detail in an earlier paper (4). It includes enzyme hydrolysis, continuous ether extraction at pH 1, and column chromatography in the gradient elution system of Lakshmanan and Lieberman (5). The peaks of Zimmermann material obtained in this fashion were analyzed for their content of unsaturated analogs by the method of Bitman *et al.* (6). No effort was made to separate the 11-oxy 17-ketosteroids further. The amounts of 11-OH androsterone, 11-ketoetiocholanolone and other peaks of Zimmermann material appearing in the characteristic area of the chromatogram plus the calculated values of delta-9 artefacts are listed under the general heading of 11-oxy 17-ketosteroids. The identity of androsterone and etiocholanolone was established in periodic samples by infrared analysis.

RESULTS

Patient B, a 70-year-old male, was admitted for subtotal gastrectomy (peptic ulcer) and cholecystectomy (chronic cholecystitis). The operation was performed on day 16 of the study period. Surgery was uneventful, but a staphylococcal wound infection of severe magnitude developed. In the immediate postoperative period a decrease in androsterone and etiocholanolone excretion was observed. This was associated with a rise in 11-oxy 17-ketosteroid excretion. On days 43, 44 and 45 following surgical inter-

TABLE 1. URINARY 17-KETOSTEROID EXCRETION (MG./24 HRS.)—PATIENT B

	Preop.	Postop. (day 1–2)	Postop. (days 43, 44 & 45)
Androsterone	2.0	0.6	0.9
Etiocholanolone	2.6	2.1	1.2
11-Oxy 17-KS	5.2	6.5	4.6

vention, when the patient had not fully recovered, it was evident that further depression of the urinary 17-ketosteroids, except for androsterone, had occurred (Table 1).

Patient A, a 29-year-old male with a colostomy previously established because of a gunshot wound in the abdomen, was admitted for closure of the colostomy (day 10 of study) and bowel resection. The patient was in fair health at the time of admission. Operation and recovery were entirely uneventful. The urinary excretion of androsterone and etiocholanolone fell in the immediate postoperative period in association with a sharp rise in the excretion of 11-oxy 17-ketosteroids. At the end of the study, when the patient was completely recovered, the excretion of androsterone and etiocholanolone was above the presurgical level. The excretion of 11-oxy 17-ketosteroids was less than immediately after surgery, but was above the admission level (Table 2).

Patient H, a 32-year-old male, was admitted for subtotal gastrectomy (peptic ulcer), was which carried out on day 10 of study. Operation and recovery were uneventful. In this case also, urinary androsterone and etiocholanolone excretion fell and 11-oxy 17-ketosteroid excretion rose slightly in the immediate postoperative period. No studies were carried out in the latter part of the recovery perod (Table 3).

Patient C, a 30-year-old male, was admitted for repair of an inguinal hernia. He fasted for thirty-six hours before the operation, which was performed on day 10 of study. Operation and recovery were entirely unevent-

TABLE 2. URINARY 17-KETOSTEROID EXCRETION (MG./24 HRS.)—PATIENT A

	Preop.	Postop. (day 1–2)	Postop. (days 28, 29 & 30)
Androsterone	2.9	2.6	6.6
Etiocholanolone	2.2	1.5	3.3
11-Oxy 17-KS	3.3	8.8	7.4

TABLE 3. URINARY 17-KETOSTEROID EXCRETION (MG./24 HRS.)—PATIENT H

	Preop.	Postop. (day 1–2)
Dehydroisoandrosterone	.1	.1
Isoandrosterone	.6	.6
Androsterone	3.1	1.4
Etiocholanolone	2.2	1.5
11-Oxy 17-KS	3.2	3.5

ful. Following the preoperative fasting period, there was a decrease in the urinary excretion of all C-19 O_2 steroids; this was further accentuated by surgical stress. A return to preoperative values was again seen as clinical recovery progressed (Table 4).

The urinary excretion data on 5 medical students (males) who were given intravenous infusions of ACTH are presented in Table 5. They indicate a uniform rise for all C-19 O_2 and O_3 steroids.

TABLE 4. URINARY 17-KETOSTEROID EXCRETION (MG./24 HRS.)—PATIENT C

	Resting	Fasting	Postop. (day 1–2)	Postop. (day 18)
Dehydroisoandrosterone	1.6	.2	.3	2.7
Isoandrosterone	3.0	.9	.7	2.3
Androsterone	4.1	2.9	1.9	2.5
Etiocholanolone	4.1	2.8	2.4	5.3
11-Oxy 17-KS	4.1	4.5	4.2	4.2

TABLE 5. URINARY 17-KETOSTEROID EXCRETION (MG./24 HRS.) IN 5 NORMAL MALES BEFORE AND AFTER ACTH

Subject	Before ACTH				After ACTH			
	DHIA	Andro.	Etio.	11-Oxy 17-KS	DHIA	Andro.	Etio.	11-Oxy 17-KS
A.T.	.3	2.7	4.7	1.8	8.9	9.4	11.2	8.5
C.L.	.1	1.2	1.2	2.6	.8	2.8	2.4	7.7
K.A.	3.0	3.1	5.3	5.0	8.3	4.3	5.3	10.2
K.I.	1.8	1.0	2.9	2.1	7.3	2.1	5.8	8.7
McK.	3.3	2.9	5.7	3.4	10.7	5.5	8.1	11.5

Patients B, A, H and C all showed a decrease in the excretion of C-19 O_2 steroids associated with a variable increase in the excretion of 17-ketosteroids carrying an oxygen function in carbon 11. The latter observation is not surprising, since these compounds are known to derive, at least in part, from C-21 compounds. The increase following operation is therefore comparable to the findings of other investigators concerning the response of corticoids to trauma. The diminished excretion of androsterone and etiocholanolone following operation is of great interest, particularly when one compares these data with those obtained following administration of ACTH, when a two- to threefold increase of these steroids is observed. It has been shown before that the excretion of androsterone and etiocholanolone for a specific individual is remarkably constant (5, 6). This, and the fact that our determinations were made on large specimens of pooled urine make random variation unlikely. It is also apparent from this study that the changes cannot be explained by ACTH stimulation alone. Presumably, stress influences adrenal steroid metabolism by other means than ACTH stimulation. One can speculate that in order to preserve the homeostatic milieu, the synthesis of C-19 O_2 precursors is decreased in the presence of accelerated synthesis of cortisone-like material. A second but less appealing possibility would be a differential change in the pathways of catabolism and excretion of these compounds.

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Acknowledgment

The authors wish to express their gratitude to Miss Fritze Pedersen and Miss Viebeke Nielsen for their competent technical assistance.

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THE INTERPRETATION OF STEROID HALF-LIFE VALUES

TO THE EDITOR:

In a Letter to the Editor in the October 1958 issue of this journal, data were presented concerning the half-life of plasma free 17-hydroxycorticosteroids (17-OHCS) following the injection of hydrocortisone hemisuccinate at various ages including the first day of life (1). We have made similar studies in newborn infants and adults, using the free alcohol form of hydrocortisone. Although our results indicate that half-life determinations can be significantly influenced by a number of factors we should like to comment on only one of these, *viz*, the dose of administered steroid. To stay within the limits of design of a Letter to the Editor, discussion of other points will be reserved until our data are published.

Although there is evidence that in adults hydrocortisone free alcohol is removed from the circulation in accordance with a first-order reaction over a wide range of doses and that the dose has no significant effect on the half-life value (2), this has not been shown to be true in infants and children. Other evidence suggests that higher plasma concentrations of cortisol result in a higher percentage recovery of tracer doses of chloroform-extractable radioactive cortisol (3). In our study, when hydrocortisone free alcohol was administered in a dose of 1 mg. per Kg. of body weight there was no significant difference between the resulting 17-hydroxycorticosteroid half-life values in adults and those in premature or term infants during the first week of life. However, in newborn term and premature infants, when a comparison was made between hydrocortisone free alcohol doses of 1 mg.

per Kg. and 2 mg. per Kg., the 17-OHCS half-life values resulting from the higher dosage were found to be significantly ($P<.01$) greater. It would be of great interest to know what half-life values would result from even higher doses and from doses less than 1 mg. per Kg. When half-life values in term and premature infants were compared on the basis of a dose of 2 mg. per Kg., no significant difference was found. These data suggest that at least in newborn infants the dose of administered steroids can influence significantly the resulting half-life values. In our study the *total* amounts of hydrocortisone free alcohol that were administered did not exceed 7 mg., and since the larger dose resulted in a significant prolongation of cortisol half-life, still larger doses, *i.e.*, 25 mg. (approximately 8 mg. per Kg. in an infant weighing 7 pounds) could be expected to prolong the half-life even more. This may account partially for the differences between our findings and those of Bongiovanni *et al.* (1). Because these investigators administered a dose of 25 mg. of hydrocortisone hemisuccinate to all of the subjects that they studied who were under the age of 1 year, their dose *per kilogram* necessarily would vary from approximately 8 mg. in a 7-pound infant to 2.6 mg. in a 21-pound infant (an approximate average weight at 1 year of age). In the subjects over 2 years of age to whom doses of 50 mg. were administered by these investigators, the dose per kilogram again would vary; the average 2-year-old subject probably would have received about 4 mg. per Kg. As adult weights were approached the dose per kilogram would decline to 1 mg. in a 50-Kg. person and to 0.7 mg. in one weighing 70 Kg.

As to the form of hydrocortisone that is administered, there is evidence that when hydrocortisone free alcohol and hydrocortisone hemisuccinate are administered intravenously to dogs (4) and to adult human subjects (5) in a dose of 1 or 2 mg. per Kg., the half-life of plasma free 17-hydroxycorticosteroids following the injection of hydrocortisone hemisuccinate is not significantly longer than that following the injection of hydrocortisone free alcohol. Whether this would also hold for doses of the magnitude of 8 mg. per Kg. would appear to be a question of great importance. Since hydrocortisone hemisuccinate first is de-esterified to the free alcohol form and the latter then is metabolized, it is possible that in the newborn (because of relatively immature hepatic function or other reasons) an extended period of time might be required to accomplish the de-esterification of large amounts of hydrocortisone hemisuccinate esters. Were this true, the effect produced could be similar to that of a continuous infusion of hydrocortisone free alcohol; and if the plasma specimens were obtained at post-infusion times that were insufficient in length to compensate for this proposed prolonged de-esterification, the resulting half-life of plasma free 17-OHCS might be artefactually prolonged. If this hypothesis be cor-

rect, we may have a further explanation for the extremely long half-life values that were observed.

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The Endocrine Society

RECIPIENTS OF THE 1959 AWARDS

THE FRED CONRAD KOCH AWARD OF THE ENDOCRINE SOCIETY

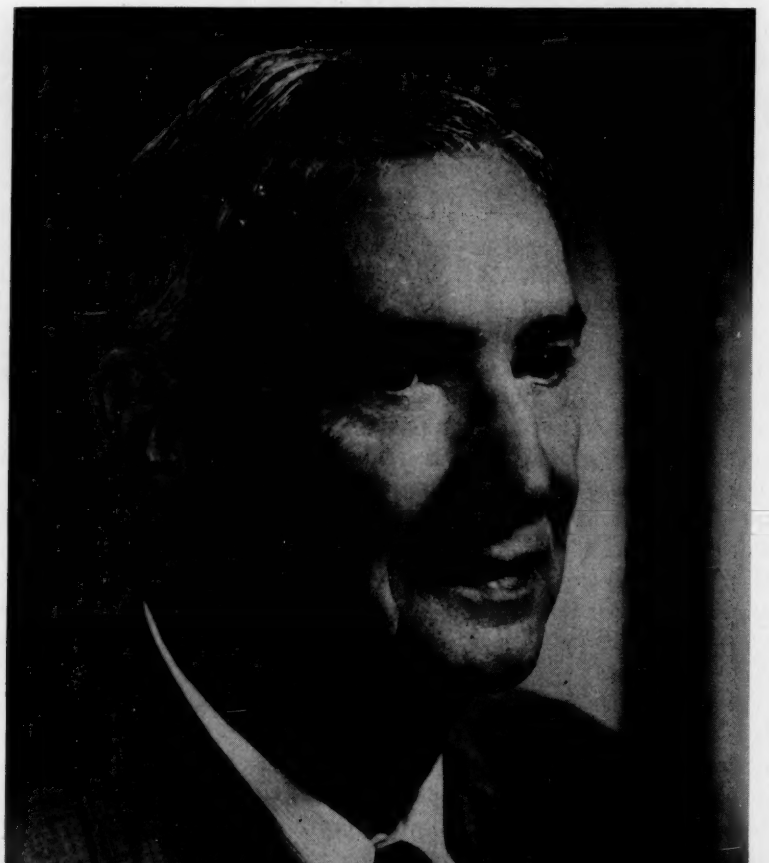
The Fred Conrad Koch Award of the Endocrine Society is the highest award made by the Society and is bestowed on an individual who has made distinguished contributions to endocrinology. This year it is awarded to *Dr. Wilbur Willis Swingle*, Henry Fairfield Professor of Biology in Princeton University.

Dr. Swingle received his B.A. and M.A. degrees at the University of Kansas and his Ph.D. degree in biology from Princeton University. He then joined Professor Ross Harrison's department at Yale University, first as instructor, then as assistant professor. In 1926 he was appointed Professor and Chairman of the Department of Biology at the University of Iowa. His stay at Iowa, however, was a brief one, for in 1929 he was called to Princeton University as Professor of Biology. He served the University with distinction as the Conklin Professor of Biology until, having reached the statutory retiring age, he was appointed to the research professorship he now holds.

Throughout his scientific career Dr. Swingle and his numerous colleagues and collaborators have devoted themselves to the study of the function of the endocrine organs. His first paper published in 1917 dealt with the effects of feeding thymus gland to frog larvae and a perusal of his list of publications reveals that, with very few exceptions, the 150 papers appearing under his name have reported the results of his studies on a variety of topics in endocrinology.

His name, however, will always be associated with our knowledge of the adrenal cortical hormones. His first paper on adrenal cortical function was published in 1926 and in this and several subsequent contributions he described the occurrence of hemoconcentration, acidosis, increased blood urea content and hypoglycemia in bilaterally adrenalectomized cats. It is quite evident that these early studies on the profound chemical changes in the blood of animals dying in adrenal insufficiency had a marked influence on his subsequent work and one may surmise, turned his thoughts to the possibility of isolating the active principle of the adrenal glands. Others

THE FRED CONRAD KOCH AWARD OF THE ENDOCRINE SOCIETY



DR. W. W. SWINGLE

before Swingle had attempted to obtain this active principle. Hartman as well as Stewart and Rogoff had reported that saline extracts of the adrenal prolonged the life of adrenalectomized animals, but none had succeeded in obtaining extracts that restored adrenalectomized animals to full health.

In the last week of 1929 at the meeting of the American Society of Zoologists held at Ames, Iowa, Swingle and his colleague Pfiffner reported that they had prepared an extract from the *lipid* fraction of beef adrenals that greatly prolonged the life of adrenalectomized cats. Early in 1930 they were able to state that such extracts would maintain adrenalectomized animals in perfect health for an indefinite period and that withdrawal of the extract was followed by death in typical adrenal insufficiency within a few days. By one of the coincidences that are often associated with a major discovery, Hartman and Brownell in the same journal also reported that a lipid extract was far more effective than water or saline extracts in supporting adrenalectomized animals.

These papers mark a turning point in the history of our knowledge of adrenal cortical function since they demonstrated not only that it was possible to concentrate the active principle of the gland, but what was equally important, that this principle was associated with the lipids of the gland. This clue was soon followed up and by 1937 several laboratories, particularly those of Reichstein, Kendall and Wintersteiner, had not only isolated active crystalline materials from such lipid extracts, but had identified the active principles as belonging to the steroid group of compounds.

Swingle, however, did not pursue the chemistry of these new principles but instead began an investigation on the nature of their control of bodily functions. No doubt he had been impressed by the profound changes in water and salt metabolism that he and others had observed to follow adrenalectomy, and he and his collaborators soon demonstrated that the administration of the new adrenal extracts rapidly reversed this disequilibrium. In a long series of papers he analyzed these water and electrolyte changes and reached a conclusion that he still holds, that a major function of the adrenal hormones is to facilitate the passage of water and salts between the fluid compartments of the body. Although others have shown that certain adrenal steroids such as desoxycorticosterone and aldosterone probably have a specific effect on electrolyte transfers in the renal tubules, Swingle demonstrated in a most ingenious series of experiments that renal sodium loss and consequent reduced sodium concentration in the extracellular fluid was not in itself entirely responsible for the disordered water and electrolyte metabolism observed in adrenal insufficiency. Indeed in his most recent papers he draws a clear distinction between the renal effects produced by aldosterone and the water shifts between the body compartments produced by cortisone and its allied compounds.

The adrenal gland has always been notorious for the controversies that have raged about its function. It will be recalled that long before much attention was paid to the cortical portion of the gland, the nature of the medullary secretion had aroused a heated debate. Soon after the introduction of potent adrenal extracts, controversy also arose as to the prepotent function of the adrenal cortex. Many of you will remember the often sharp differences of opinion between Dr. Swingle and his colleagues on the one hand, and Dr. Britton and his collaborators, as to the primary effect of these hormones. As has been stated, Swingle from his studies emphasized the importance of the control of water and salt metabolism, whereas Britton from his work inclined to the view that it was the evident disturbances in carbohydrate metabolism of adrenalectomized animals that were to be considered as an indication of the major site of action of these hormones.

Although we are still far from an understanding as to the manner by which a single hormone can influence both electrolyte and water transfers as well as carbohydrate and protein metabolism, nonetheless we now know that the observations of both laboratories were correct and, as is often the case in scientific controversy, it was the interpretation of the evidence rather than the evidence itself that was the cause of the differing opinions.

The measure of a scientist is to be found not only in his own contributions to knowledge but also in the number and distinction of his pupils. Dr. Swingle has fulfilled both these criteria to an admirable degree. Up to the present time no less than 38 pupils have completed the work for the Ph.D. degree in his laboratory. Many of these hold professorial positions, or other positions of importance in the research laboratories of government or industry. The record of their achievement must be a great source of satisfaction to their old chief, and their continued loyalty and admiration of him must be as proud a distinction as that which The Endocrine Society confers on him this evening.

The Endocrine Society is happy to present to Dr. Swingle the first Fred Conrad Koch Award. It does so in recognition of his contributions to endocrinology, not only as an investigator but also as a great teacher who has been the inspiration of so many students who, in their turn, have broadened and enriched the subject that has been his life work.

THE AYERST FELLOWSHIP

DR. HENRY ANGUS BORIGHT

The recipient of the Ayerst Fellowship for 1959 is *Dr. Henry Angus Boright.* Dr. Boright was born in Montreal, Canada, on March 15, 1929, and obtained his collegiate training at McGill University. He was granted the B.Sc. degree in 1951 and the M.D. in 1955. During the period 1956–1957 he was an interne in medicine at the Royal Victoria Hospital, Montreal. The following year, 1957–58, he was occupied as a research fellow in the Endocrine-Metabolic Unit at the Royal Victoria Hospital, studying the relationship of serum mucopolysaccharides to the development of renal disease in diabetes. This project provided the material for a thesis towards the M.Sc. degree, and also two publications. During the past year he has been an assistant resident in the Department of Medicine at the Royal Victoria Hospital.

Dr. Boright now will leave the cold of Montreal to spend a year in the warmth of North Carolina, where he will work in the laboratories of Dr. Frank Engel at Duke University. The studies will be concerned with the relationship of growth hormone to fat metabolism in experimental animals.

DR. GORDON L. FARRELL

THE CIBA AWARD

The Ciba Award for 1959 is made to *Dr. Gordon L. Farrell* for outstanding contributions in the field of ACTH and the adrenal cortical hormones.

Dr. Farrell was born on April 16, 1925. He attended Carroll College in Helena, Montana, 1940–43, and received the M.D. degree in 1947 at St. Louis University. He then held the following positions: Intern, U.S.P.H.S. Hospital, New Orleans, 1947–48; Assistant Surgeon, U.S.P.H.S., 1948–1951; Instructor, Department of Pharmacology, University of Utah, 1951–52; Research Fellow, Department of Physiology, Western Reserve University, 1952–54; instructor, 1954–56; and Assistant Professor, 1956 to date.

Dr. Farrell was the first to demonstrate that desoxycorticosterone and

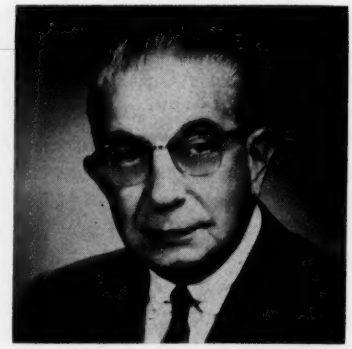
substance S are secreted by the adrenal cortex. These studies were followed by the isolation and characterization of aldosterone from the adrenal venous blood of the dog.

The application of quantitative procedures to the detection of ACTH in blood demonstrated that epinephrine increased the level in rat blood to a detectable amount and long-term administration of cortisone inhibited the synthesis of ACTH at the pituitary level. The extension of these studies to hypophysectomized animals yielded an understanding of the time relationship between ACTH release and steroid synthesis in the adrenal cortex. A comparison of the ability of various ACTH preparations to stimulate adrenal cortical secretion indicated that separate factors were present for the secretion of aldosterone and cortisol.

The physiologic factors which regulate aldosterone secretion have been studied intensively by Dr. Farrell during the past few years. Investigations of the effects of hypophysectomy, central nervous system ablations and brain-stem lesions led to the discovery that areas important in aldosterone regulation exist in the posterior diencephalon and anterior mid-brain. This property has been demonstrated to be due to a new hormone which can be extracted from the diencephalon or the pineal complex. Dr. Farrell has given the hormone the conditional name of "glomerulotropin."

His more recent studies have been concerned with the influence of changes in blood volume, of heart failure and of alteration in electrolyte intake on the rate of aldosterone secretion.

Dr. Farrell now is extending his basic investigations and applying this new knowledge to studies on patients with aldosterone-secreting tumors and patients with hypertension.



DR. HENRY H. TURNER

THE SCHERING SCHOLAR

Dr. Henry Hubert Turner was born in Harrisburg, Illinois. He was educated in Harrisburg, at St. Louis University, and received an M.D. degree from the University of Louisville School of Medicine. He took postgraduate training at the University of Vienna and in Queen's Square, London.

Henry, affectionately known to his close intimates as Jack, has been prominent in American medicine for many years. He became Associate Professor of Medicine at the University of Oklahoma School of Medicine in 1939, and in 1949 was made Clinical

Professor of Medicine. He is widely known as a lecturer, especially in endocrinology, before medical societies in various parts of the country and at many postgraduate courses of the American College of Physicians and the postgraduate assemblies of the Endocrine Society. He is a Diplomate of the American Board of Internal Medicine, President of The Society of Nuclear Medicine, and a member of many other medical societies.

He is perhaps most widely known for his paper entitled "A Syndrome of Infantilism, Congenital Webbed Neck, and Cubitus Valgus" published in *Endocrinology* in 1938. This is a subject on which he has lectured and written since and has modestly called ovarian agenesis but which most others know as "Turner's syndrome." He has been an active member of The Endocrine Society for many years and has been its hard-working Secretary since 1941. His present interest is a follow-up study of testicular histology and physiology especially in relationship to former treatment with chorionic gonadotropin. The award of the Schering Scholarship will make it possible for him to visit various endocrine centers in this country and abroad in which he can gather further information in the line of his present investigation.

THE UPJOHN SCHOLAR

DR. HAROLD L. MASON

The Upjohn Scholar for 1959 is *Dr. Harold L. Mason.* Dr. Mason has had a distinguished career in steroid endocrinology. He collaborated with Dr. E. C. Kendall in the pioneer studies on the isolation, characterization and synthesis of the adrenal cortical steroids.

He then turned his acquired skill in steroid chemistry to the isolation from the urine of patients of many hitherto unrecognized metabolites of not only the adrenal cortical steroids but also of the androgens. More recently he tackled the question of the active material in the amorphous fraction and announced the successful isolation of aldosterone only shortly after the report of the European team of investigators.

Dr. Mason was born in Compton, California, April 17, 1901. He earned the B.A. degree at the University of Southern California in 1923, the A.M. degree in 1924, and the Ph.D. (Organic Chemistry) degree in 1927 from the University of Chicago. He was an instructor in chemistry at Northwestern University, 1927–28. In 1928 Dr. Mason was appointed Professor of Physi-

ological Chemistry at the Mayo Foundation and became Head of the Department after Dr. Kendall's retirement.

The scholarship will be used by Dr. Mason to carry out some collaborative work at the Worcester Foundation for Experimental Biology and the National Institutes of Health. The time spent at these two institutions is certain to benefit Dr. Mason and probably will bring even greater benefit to the laboratories.

THE 1960 ANNUAL MEETING

The Forty-Second Meeting of The Endocrine Society will be held in the Eden Roc Hotel at Miami Beach, Florida, *June 9–11, 1960.*

HENRY H. TURNER, M.D., *Secretary,*
1200 North Walker Street,
Oklahoma City 3, Oklahoma

THE 1960 AWARDS

The selection of the recipients of the awards of The Endocrine Society is made by a Committee appointed by Council of the Society. These awards and fellowships carry no obligation by the recipient to the Society or to the donors.

FRED CONRAD KOCH AWARD

In 1957 a substantial legacy was bequeathed to the Society by the late Elizabeth Koch for the purpose of establishing the Fred Conrad Koch Memorial Fund in memory of her late husband, Distinguished Service Professor of Physiological Chemistry at the University of Chicago and pioneer in the isolation of the androgens. This is the highest honor of The Endocrine Society and is represented by a medal known as the Koch Medal of The Endocrine Society. The medal, as well as an honorarium of $3,500, is to be given annually to an individual for work of special distinction in endocrinology. The recipient shall be chosen from nominations presented by members of the Society and is limited to citizens of the United States and Canada.

This Award was first presented in 1959, to Dr. W. W. Swingle. The Koch Medal has replaced the Medal of The Endocrine Society which was established in 1954 and presented to Dr. Carl R. Moore in 1955, Dr. Frederick L. Hisaw in 1956, Dr. Joseph C. Aub in 1957 and Dr. I. L. Chaikoff in 1958. The Medal of the Endocrine Society replaced the E. R. Squibb Award which was formerly the highest honor bestowed by the Society.

Past recipients of the Squibb Award were Dr. George W. Corner in 1940, Dr. Philip E. Smith in 1941, Dr. Fred C. Koch in 1942, (no award in 1943), Dr. E. A. Doisy in 1944, Dr. E. C. Kendall in 1945, Dr. Carl G. Hartman in 1946, Drs. Carl F. and Gerty T. Cori in 1947, Dr. Fuller Albright in 1948, Dr. Herbert M. Evans in 1949, Dr. C. N. H. Long in 1950, Dr. J. B. Collip in 1951, Dr. James H. Means in 1952 and Dr. David Marine in 1953.

THE CIBA AWARD

The Ciba Award, to recognize the meritorious accomplishments of an investigator not more than 35 years of age in the field of clinical or preclinical endocrinology, was established in 1942, but no recipient was selected in 1942 or 1943. In 1944 the Award was presented to Dr. E. B. Astwood; 1945—Dr. Jane A. Russell; 1946—Dr. Martin M. Hoffman; 1947—Dr. Choh Hao Li; 1948—Dr. Carl Heller; 1949—Dr. George Sayers; 1950—Dr. Oscar M. Hechter; 1951—Dr. Albert Segaloff; 1952—Dr. Seymour Lieberman; 1953—Dr. Sidney Roberts and Dr. Clara Szego (Mrs. Roberts); 1954—Dr. Isadore M. Rosenberg; 1955—Dr. Jack Gross; 1956—Dr. Alfred M. Bongiovanni; 1957—Dr. Nicholas S. Halmi; 1958—Dr. Monte A. Greer; 1959—Dr. Gordon L. Farrell. Prior to 1952 the Award was $1,200. It has now been increased to $2,500.

THE AYERST AND THE SQUIBB FELLOWSHIPS

The Ayerst Fellowship was established in 1947 and the Squibb Fellowship in 1956. They are designed to assist men or women of exceptional promise in furthering their advancement towards a career in endocrinology. Each Fellowship is awarded on alternate years and the stipend, which will not exceed $5,000, may be divided into two Fellowships in varying amounts in accordance with the qualifications of the appointees. Individuals possessing the M.D. or Ph.D. degree, or candidates for either of these degrees, are eligible for appointment.

Applicants must submit the following information:

1. Evidence of scientific ability as attested by studies completed or in progress.
2. Recommendations from individuals familiar with the candidate and his work.
3. A proposed program of study.
4. Acceptance of the individual by the head of the department in which the Fellowship will be held.
5. A statement that he or she will serve full time if awarded a Fellowship. A small amount of time (10 to 15 per cent) may be spent in course work or participation in teaching, the latter purely on a voluntary basis.

THE UPJOHN SCHOLAR OF THE ENDOCRINE SOCIETY

The Council of The Endocrine Society has established a category of Scholars. A grant has been made available through the generosity of the Upjohn Company, and will be awarded to an established investigator or teacher in the field of endocrinology who wishes to extend the opportunities for work either in this country or abroad.

The award will not exceed $2,500 annually and will be granted on the basis of proposals submitted by the applicant. Such applications should include the estimated financial needs. The funds may be used for travel, maintenance and other expenses.

Nominations

Nominations for the Fred Conrad Koch Award, the Ciba Award, and the Ayerst and the Squibb Fellowships may be made by any member of The Endocrine Society. They should be submitted on forms which may be obtained from the Office of the Secretary, 1200 North Walker Street, Oklahoma City 3, Oklahoma. Completed nominations should be returned to the Secretary not later than *October 15* each year.

Proposals for appointments as a Scholar of The Endocrine Society should be made in writing by the individual, and addressed to the Secretary of the Society. They should be submitted by *October 15* each year.

ELEVENTH POSTGRADUATE ASSEMBLY IN ENDOCRINOLOGY AND METABOLISM

SPONSORED JOINTLY BY THE ENDOCRINE SOCIETY AND THE UNIVERSITY OF CALIFORNIA SCHOOL OF MEDICINE AND DEPARTMENT OF CONTINUING MEDICAL EDUCATION

SAN FRANCISCO, CALIFORNIA

November 9 through 13, 1959

For information concerning the program and registration, write to *Seymour M. Farber, M.D.*, Assistant Dean in charge of Continuing Medical

(For program, see the June issue of the Journal)

Education, University of California Medical Center, San Francisco 22, California.

The registration fee is $100. Residents and Fellows will be admitted for a reduced fee of $30. Those wishing to reserve rooms should communicate directly with the Whitcomb Motor Hotel, 8th and Market St., San Francisco.

FIRST INTERNATIONAL CONGRESS OF ENDOCRINOLOGY

The First International Congress of Endocrinology will be held in *Copenhagen, Denmark, July 18–23, 1960.*

United States members of the International Executive Committee are Dr. Warren O. Nelson and Dr. Leo T. Samuels, Dr. Gregory Pincus is Chairman of the Program Committee. The tentative program consists of ten symposia covering the general field of endocrinology plus round-table discussions and a number of submitted papers. Those desiring to submit papers should immediately contact Dr. Gregory Pincus, Worcester Foundation, Shrewsbury, Massachusetts. There will be a number of interesting scientific and technical exhibits.

In order to facilitate travel arrangements to the Copenhagen Convention, the Council of The Endocrine Society at its San Francisco meeting designated THOMAS COOK & SON as our authorized travel agents. They will in turn offer especially designed All Expense Paid Tours to Europe, including hotel reservations for the convention, as well as offering independent itineraries for any of our members who desire to travel separately by air or by steamship.

Registration is open to anyone interested in endocrinology at an approximate fee of $28 U.S. Members of the families of those attending may register as associates at an approximate fee of $14 U.S., fee to be paid at the time of registration or in advance to the American Express Company, which has been designated by the Executive Committee of the International Congress as official financial and travel agent. There will be a number of social functions and other entertainment provided for all registrants.

As you all know, European travel is unusually heavy during July and it is suggested that those contemplating attending the Congress should start making tentative reservations at the earliest possible moment by filling in

and returning the card recently mailed to members, to THOMAS COOK & SON's special representative—Harry E. Kornbaum, Transportation Chairman, Rainbow Travel Service, 2817 Classen Boulevard, Oklahoma City 6, Oklahoma, who will correspond directly with each member about his individual travel arrangements to and from the Convention, at the same time keeping our National Officers fully informed of all travel arrangements of its members. Completion of the card CARRIES NO OBLIGATION OR COMMITMENT ON YOUR PART. Send it in today for the complete travel arrangements for our Society.

The general expenses of a meeting of this magnitude are quite large and The Endocrine Society has made a substantial contribution from its treasury for organizational purposes. U.S. pharmaceutical firms have also contributed very generously. However, sufficient funds are not as yet available to assure the success of this meeting; therefore, *the committee suggests that each of our members make voluntary contributions to this fund.* Please make your checks payable to: The Endocrine Society—First International Congress of Endocrinology. Mark it *Contribution* and mail it to the office of the Secretary-Treasurer, 1200 North Walker Street, Oklahoma City 3, Oklahoma.

PROGRAM

The program will consist of ten Symposia and a Round Table Discussion, and of groups of shorter (ten-minute) communicated papers. Speakers at the Symposia and the Round Table Discussion are invited by the Congress. Communicated papers may be submitted by investigators in the field of endocrinology, and titles and abstracts are invited. Forms for registration in the Congress, along with forms for the submission of abstracts of short communications, may be obtained by writing to the Congress Secretariat: Dr. Svend G. Johnsen, Hormone Department, Statens Seruminstitut, Copenhagen S, Denmark. The final date for the submission of such abstracts is *December 31, 1959.* The official languages of the Congress are English, French, German and Spanish. A volume containing advance abstracts of symposium contributions and the short communications (in one of the official languages and translated into Interlingua) will be distributed to members on registration at the Technical University of Denmark in Copenhagen. Titles and abstracts of communicated papers should not be sent to the Program Committee, but the Program Committee will review and arrange for the presentation of such papers in appropriate Congress sessions.

GREGORY PINCUS, *Chairman,*
Subcommittee on Program

ACKNOWLEDGMENT

The Editorial Board of THE JOURNAL OF CLINICAL ENDOCRINOLOGY AND METABOLISM wishes to acknowledge with gratitude the generous and expert aid of the following reviewers:

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In addition, H. L. Mason has on numerous occasions given wise counsel and excellent advice on manuscripts concerned with steroid chemistry.

A. ALBERT
Editor-in-Chief

The American Goiter Association

RECIPIENTS OF THE 1959 AWARDS

THE VAN METER PRIZE AWARD

Dr. Takashi Yamada has been named the winner of the Van Meter Prize Award for 1959. The title of his paper was "Studies on the Mechanism of Hypothalamic Control of Thyrotropin Secretion in the Rat."

Dr. Yamada was born in Sado, Niigata Prefecture, Japan, on May 1, 1926. His premedical education was at Toyama University. After teaching school for two years, he studied medicine at Gunma University where he received his M.D. degree in 1954. After serving an internship of one year at Tokyo First National Hospital, he returned to Gunma University to work in the field of neuro-endocrinology where his studies led to a Ph.D degree. Since September 1957, he has been a research fellow in the Division of Endocrinology at the University of Oregon Medical School, working under the direction of Dr. Monte A. Greer.

AWARD FOR LONG AND MERITORIOUS SERVICE

The certificate awarded by the American Goiter Association for Long and Meritorious Service to the Association was presented to *Rulon W. Rawson, M.D.*, Professor of Medicine of the Sloan-Kettering Division of Cornell University Medical College. This award of distinction has been presented annually to members of the Association for contributions to both the scientific and organizational function of the Association.

Doctor Rawson has served on the Council of the Association, has been Chairman of many of its committees and was President in 1955–1956. Each year he and his associates have had one or more papers on the scientific program, many of which have dealt with the basic physiology of the thyroid gland. Because of his clinical experience in addition to research activities, he has frequently been called upon to correlate pure research information presented at the annual meetings with the clinical application of this newly acquired knowledge.

Born in Utah in 1908, Doctor Rawson came to Northwestern University for his medical degree and early post-graduate training. He achieved international recognition for his work in the Thyroid Clinic of the Massachusetts General Hospital in Boston, where he also served on the faculty

of Harvard University. Since 1948 he has been affiliated with Cornell University College of Medicine at its Sloan-Kettering Division, in both of which he holds the title of Professor of Medicine. He directs the Division of Clinical Investigation of the Sloan-Kettering Institute.

Over the years, more than 125 papers have been published bearing Doctor Rawson's name together with those of his co-workers. For this contribution to the knowledge of the thyroid gland and its function in health and disease, he ranks with those gifted few throughout the world who have contributed the most in this field.

THE 1960 ANNUAL MEETING

The next Annual Meeting of the American Goiter Association will be held in *London, England, July 5–9, 1960,* in the Great Hall of the Royal College of Surgeons. This meeting, co-sponsored with the London Thyroid Club, will be the Fourth International Goiter Conference. For further information write Dr. Selwyn Taylor, 3 Roedean Crescent, Roehampton, London, S.W.3, England.

JOHN C. MCCLINTOCK, *Secretary,*
149½ Washington Avenue,
Albany 10, N.Y.

SUBMISSION OF ABSTRACTS FOR THE PROGRAM OF THE FOURTH INTERNATIONAL GOITER CONFERENCE

Abstracts of American papers to be considered for presentation at the Fourth International Goiter Conference, London, England, July 5–9, 1960, should be sent to Dr. J. E. Rall, National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda 14, Maryland. Abstracts from all other countries are to be sent to Dr. Selwyn Taylor, 3 Roedean Crescent, Roehampton, London, S.W.3, England.

All American abstracts must be received by Dr. Rall *by December 1, 1959.* They should not exceed 400 words and should be submitted in quintuplicate. Presentation of papers will be limited to fifteen minutes and a copy of the final paper must be sent to the London Secretary two weeks prior to the meeting.

THE VAN METER PRIZE AWARD FOR 1960

The American Goiter Association again offers the Van Meter Prize Award of $300.00 to the essayist submitting the best manuscript of original and unpublished work concerning "Goiter—especially its basic cause."

The studies so submitted may relate to any aspect of the thyroid gland in all of its functions in health and disease. The Award will be made at the Fourth International Goiter Conference in London, England, July 5–9, 1960, where a place on the program will be reserved for the winning essayist if he can attend the meeting. For 1960, the recipient of the Award will receive consideration for an award of a travel honorarium.

The competing essays may cover either clinical or research investigations, should not exceed 3,000 words in length and must be presented in English. Duplicate typewritten copies, double spaced, should be sent to the Secretary, Dr. John C. McClintock, 149½ Washington Avenue, Albany 10, New York, *not later than January 1, 1960.* The committee who will review the manuscripts is composed of men well qualified to judge the merits of the competing essays.

UROLOGY AWARD

The American Urological Association offers an annual award of $1000 (first prize of $500, second prize of $300 and third prize of $200) for essays on the result of some clinical or laboratory research in urology. Competition is limited to urologists who have been graduated not more than ten years, and to hospital internes and residents doing research work in urology. The first prize essay will appear on the program of the forthcoming meeting of the American Urological Association to be held at the Palmer House, Chicago, Illinois, May 16–19, 1960. For full particulars write the Executive Secretary, William P. Didusch, 1120 North Charles Street, Baltimore, Maryland. Essays must be in his hands *before December 1, 1959.*

INTER-SOCIETY CYTOLOGY COUNCIL

The Annual Scientific Meeting of the Inter-Society Cytology Council will be held in the Statler Hilton Hotel, Detroit, Michigan, *November 19, 20 and 21, 1959.* Paul A. Younge, M.D., Secretary, 1101 Beacon Street, Brookline 46, Massachusetts.

FOURTH INTERNATIONAL SYMPOSIUM ON RADIOACTIVE ISOTOPES IN CLINICAL MEDICINE AND RESEARCH

The Fourth International Symposium on Radioactive Isotopes in Clinical Medicine and Research will take place in Bad Gastein, Austria, *January 7–10, 1960.* The topics selected for discussion will be:

1. Calcium and Strontium Isotopes in the Study of Calcium Metabolism and the Localization of Bone Tumors.

2. Peripheral Metabolism of Thyroid Hormones and Iodinated Amino Acids.
3. Radioisotopes in the Study of Glucose and Insulin Metabolism in Normal and Diabetic Patients.
4. Estimation of Total Body and Extracellular Water.
5. Estimation of Body Content and Turnover of Electrolytes.
6. Studies of Red Cell Sequestration by *in vivo* Counting.
7. Estimation of Organ Blood Flow Using Inert Gas Techniques.
8. New Developments in Radioisotope Therapy.

The official languages will be English, French and German. Those who wish to present papers should submit abstracts before the end of June 1959 to Dr. R. Höfer, Radioisotopes Laboratory, 2nd Medical University Clinic, Garnisongasse 13, Vienna 9, Austria. He will be pleased to give further detailed information.

BOOK REGISTER

Amino Acids and Peptides with Antimetabolic Activity. Ciba Foundation Symposium, edited by G. E. W. WOLSTENHOLME, M.A., B.Ch. and C. M. O'CONNOR, B.Sc. Twenty-nine participants discuss the chemical, biologic and clinical properties of amino acids and peptide derivatives. Included are contributions by Buchanan on the interference of azaserine in purine biosynthesis, Craig on bacitracin, Farber on clinical and biologic studies with actinomycins, Reilly on some aspects of azaserine, 6-diazo-5-oxo-*l*-norleucine and β-2-thienylalanine, Sheehan on the chemistry of etamycin, and Skipper on the influence of amino acid deficiencies of experimental cancer therapy; 286 pages; 28 illustrations; 1958. Little, Brown and Company, Boston, Mass. Price $8.75.

Clinical Obstetrics and Gynecology. Vol 1, No. 4 (Dec. 1958). This volume of a quarterly series contains a *Symposium on Operative Obstetrics* edited by J. ROBERT WILLSON, M.D., Professor and Head of the Dept. of Obstetrics and Gynecology, Temple University School of Medicine, Philadelphia, Pa.; and a *Symposium on Genital Cancer* edited by DANIEL G. MORTON, M.D., Professor and Chairman of the Dept. of Obstetrics and Gynecology, School of Medicine, University of California Medical Center, Los Angeles, California. There are articles by 28 contributors; 280 pages; illustrated; 1958. Paul B. Hoeber, Inc., 49 East 33rd St., New York 16, N.Y. By subscription, $18.00 for four consecutive numbers.

Haematology. British Medical Bulletin, Volume 15, Number 1, January 1959. This symposium includes 14 papers by leading British specialists in hematological research. The subjects include the hemolytic anemias, techniques involving the use of radioactive tracers in the formation and destruction of red blood cells, the absorption of iron, the role of folic acid and vitamin B_{12} in blood disorders, the biosynthesis of hemoglobin, the culture of bone-marrow cells, the epidemiology of leukemia in man, and the present-day treatment of chronic leukemia. The Medical Department, The British Council, 65 Davies Street, London W.1, England. Price $3.25.

Métabolism de l'eau dan les traumatismes du crâne. Aspects neuro-endocriniens et base du traitement hormonal. By D. PETIT-DUTAILLIS, Professeur, and E. BERNARD-WEIL, Chief, Neurosurgical Clinic, Faculty of Medicine, Paris. A Symposium presented at the 1958 annual réunion of the Society of Neurosurgery, French Language. Chapters contain a review of classic contributions to knowledge of the metabolism of water, regulation of water-electrolyte metabolism from the point of view of endocrinology, a summary and comparison of methods for management of disturbances of water metabolism in surgery and neurosurgery, and case histories illustrating the immediate and late effects of traumatism, and methods of treatment; bibliography of 154 items; 108 pages; illustrated; 1958. Masson et Cie, Éditeurs, Paris, France. Price, 1400 fr.

Social Aspects of Psychiatry. Psychiatric Research Report No. 10 of the American Psychiatric Association, edited by BENJAMIN PASAMANICK, M.D., Professor of Psychiatry, Ohio State University School of Medicine, Columbus, Ohio, and PETER H. KNAPP, M.D., Associate Research Professor of Psychiatry, Boston University School of Medicine, Boston, Mass. This volume presents the scientific papers and discussions of a Regional Research Conference held Feb. 24–25, 1958 in Columbus, Ohio, under the joint auspices of the Department of Psychiatry, School of Medicine, The Ohio State University, and the American Psychiatric Association's Committee on Research. As stated in the introduction, the proceedings demonstrate that the social scientist, however regional, is far from provincial in his contribution to psychiatric research; 208 pages; 1958. American Psychiatric Association, 1700 Eighteenth Street, N.W., Washington 9, D.C. Price $2.00.

Transactions of the Josiah Macy, Jr. Foundation Conferences. Publications Office, 16 West 46th Street, New York 36, N. Y.

Cold Injury. Fifth Conference, March 10–15, 1957, Arctic Aeromedical Laboratory, Ladd Air Force Base, Alaska. Edited by M. I. FERRER, M.D., Assistant Professor of Clinical Medicine, Columbia University College of Physicians and Surgeons, New York, N. Y. The subjects covered are: Animal Adaptation to Cold, Hibernation, Some Observations on Ventricular Fibrillation in Acute Hypothermia, The Use of Glycine in Hypothermia, Human Acclimatization to Cold, Energy Metabolism in Cold, and Avenues of Heat Loss and Peripheral Circulation; 341 pages; 73 figures; 20 tables; 1958. Price $5.95.

The Central Nervous System and Behavior. First Conference, February 23–26, 1958, sponsored by The Josiah Macy Jr. Foundation and the National Science Foundation. Edited by MARY A. B. BRAZIER, Ph.D., Neurophysiological Laboratory, Massachusetts General Hospital, Boston, Mass. The subjects covered are: Russian Contributions to an Understanding of the Central Nervous System and Behavior (A Pictorial Survey); The Nineteenth Century Background of the Russian Neurophysiologists, and Sechenov; Danilevsky, Wedensky, and Ukhtomsky; Pavlov; Bechterev; Post-Pavlovian Developments in Conditional Reflexes; Brain Stimulation and Conditional Reflexes; Electroencephalographic Studies of Conditioned Learning; and Electrical Correlates of Conditioned Learning; 450 pages; 168 figures; 1 color plate; 1959. Price $5.25.